



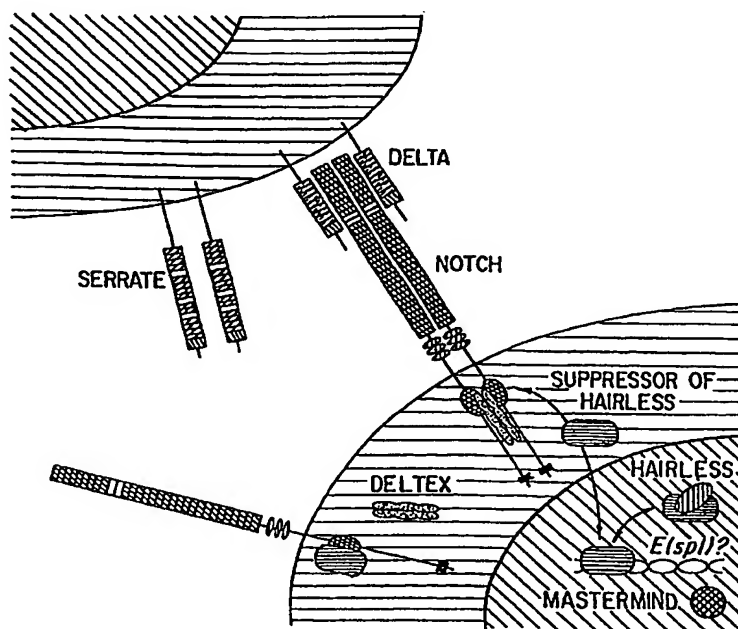
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(54) Title: DELTA CLEAVAGE PRODUCTS AND METHODS BASED THEREON

(57) Abstract

The present invention is directed to a Delta cleavage peptide and fragments, derivative and analogs thereof, and its encoding nucleic acids. The present invention is also directed to a soluble Delta peptide and fragments, derivative and analogs thereof, and its encoding nucleic acids. The present invention is also directed to protein complexes of Delta and Kuz. The present invention is also directed to methods for detecting or measuring Delta activation by observing or measuring Delta cleavage products that are indicative of Delta activation. The present invention is also directed to methods for detecting or measuring Kuz activation by observing or measuring Delta cleavage products that are indicative of Kuz activation. The present invention is also directed to methods for detecting a molecule that modulates Delta activation or Kuz function by observing or measuring a change in the amount of or pattern of Delta cleavage products. The present invention is based, at least in part, on the discovery that Delta in its active form, i.e., the form that mediates signal transduction and that binds Notch, is a soluble fragment consisting of the extracellular domain.



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DELTA CLEAVAGE PRODUCTS AND METHODS BASED THEREON

The application claims priority benefits of U.S. Provisional Application Serial No. 60/104,834, filed October 19, 1998 and U.S. Provisional Application Serial No. 60/092,513 filed July 13, 1998, each of which is incorporated by reference herein in its entirety.

1. FIELD OF THE INVENTION

10 The present invention is directed to a peptide, and its encoding nucleic acids, of the toporythmic protein Delta that contains a sequence which is cleaved by the metalloprotease-disintegrin Kuzbanian (Kuz) ("Delta cleavage peptide"), as well as derivatives and analogs thereof. The present invention is also directed to an extracellular
15 soluble peptide, and its encoding nucleic acids, of the toporythmic protein Delta ("soluble Delta peptide" or "Dl^{EC}"), as well as derivatives and analogs thereof. Production of the Delta cleavage peptide or Dl^{EC}, and derivatives, and antibodies thereto are also provided. The present invention
20 is also directed to methods for detecting or measuring Delta activation by observing or measuring Delta cleavage products that are indicative of Delta activation. The present invention is also directed to methods for detecting a molecule that modulates Delta activation by observing or
25 measuring a change in the amount or pattern of Delta cleavage products. The present invention is further directed to methods for detecting or measuring Kuz function by observing or measuring Delta cleavage products that are indicative of Kuz function. The present invention is also directed to
30 methods for detecting a molecule that modulates Kuz function by observing or measuring a change in the amount or pattern of Delta cleavage products. The present invention is also directed to certain protein complexes of Delta and Kuz and of

Dl^{EC} and Notch, and methods for their use in screening, diagnosis and therapy.

2. BACKGROUND OF THE INVENTION

5 Genetic and molecular studies have led to the identification of a group of genes which define distinct elements of the Notch signaling pathway. While the identification of these various elements has come exclusively from *Drosophila* using genetic tools as the initial guide, subsequent analyses have lead to the identification of
10 homologous proteins in vertebrate species including humans. Figure 1 depicts the molecular relationships between the known Notch pathway elements as well as their subcellular localization (Artavanis-Tsakonas et al., 1995, Science 268:225-232).

15 The *Drosophila* Notch gene encodes an ~300 kD transmembrane protein that acts as a receptor in a cell-cell signaling mechanism controlling cell fate decisions throughout development (reviewed, e.g., in Artavanis-Tsakonas et al., 1995, Science 268:225-232). Closely related homologs
20 of *Drosophila* Notch have been isolated from a number of vertebrate species, including humans, with multiple paralogs representing the single *Drosophila* gene in vertebrate genomes. The isolation of cDNA clones encoding the C-terminus of a human Notch paralog, originally termed hN, has
25 been reported (Stifani et al., 1992, Nature Genetics 2:119-127). The encoded protein is designated human Notch2 because of its close relationship to the Notch2 proteins found in other species (Weinmaster et al., 1992, Development 116:931-941). The hallmark Notch2 structures are common to all the Notch-related proteins, including, in the extracellular
30 domain, a stretch of 34 to 36 tandem Epidermal Growth Factor-like (EGF) repeats and three Lin-12/Notch repeats (LN repeats), and, in the intracellular domain, 6 Ankyrin repeats

and a PEST-containing region. Like *Drosophila* Notch and the related *C. elegans* genes *lin-12* and *glp-1* (Sternberg, 1993, Current Biology 3:763-765; Greenwald, 1994, Current Opinion in Genetics and Development 4:556-562), the vertebrate Notch
5 homologs play a role in a variety of developmental processes by controlling cell fate decisions (reviewed, e.g., in Blaumueller and Artavanis-Tsakonas, 1997, Persp. on Dev. Neurobiol. 4:325-343). (For further human Notch sequences, see International Publication WO 92/19734.)

10 The extracellular domain of Notch carries 36 Epidermal Growth Factor-like (EGF) repeats, two of which (repeats 11 and 12) have been implicated in interactions with the Notch ligands Serrate and Delta. Delta and Serrate are membrane bound ligands with EGF homologous extracellular domains, which interact physically with Notch on adjacent
15 cells to trigger signaling.

Functional analyses involving the expression of truncated forms of the Notch receptor have indicated that receptor activation depends on the six *cdcl0*/ankyrin repeats in the intracellular domain. Deltex and Suppressor of
20 Hairless, whose over-expression results in an apparent activation of the pathway, associate with those repeats.

Deltex is a cytoplasmic protein which contains a ring zinc finger. Suppressor of Hairless on the other hand, is the *Drosophila* homologue of CBF1, a mammalian DNA binding protein involved in the Epstein-Barr virus-induced
25 immortalization of B cells. It has been demonstrated that, at least in cultured cells, Suppressor of Hairless associates with the *cdcl0*/ankyrin repeats in the cytoplasm and translocates into the nucleus upon the interaction of the Notch receptor with its ligand Delta on adjacent cells
30 (Fortini and Artavanis, 1994, Cell 79:273-282). The association of Hairless, a novel nuclear protein, with Suppressor of Hairless has been documented using the yeast

two hybrid system; therefore, it is believed that the involvement of Suppressor of Hairless in transcription is modulated by Hairless (Brou et al., 1994, Genes Dev. 8:2491; Knust et al. 1992, Genetics 129:803).

5 Finally, it is known that Notch signaling results in the activation of at least certain basic helix-loop-helix (bHLH) genes within the Enhancer of Split complex (Delidakis et al., 1991, Genetics 129:803). Mastermind encodes a novel ubiquitous nuclear protein whose relationship to Notch signaling remains unclear but is involved in the Notch
10 pathway as shown by genetic analysis (Smoller et al., 1990, Genes Dev. 4:1688).

 The generality of the Notch pathway manifests itself at different levels. At the genetic level, many mutations exist which affect the development of a very broad
15 spectrum of cell types in *Drosophila*. Knockout mutations in mice are embryonic lethals consistent with a fundamental role for Notch function (Swiatek et al., 1994, Genes Dev. 8:707). Mutations in the Notch pathway in the hematopoietic system in humans are associated with lymphoblastic leukemia (Ellison et
20 al., 1991, Cell 66:649-661). Finally the expression of mutant forms of Notch in developing *Xenopus* embryos interferes profoundly with normal development (Coffman et al., 1993, Cell 73:659). Increased level of Notch expression is found in some malignant tissue in humans (International
25 Publication WO 94/07474).

 The expression patterns of Notch in the *Drosophila* embryo are complex and dynamic. The Notch protein is broadly expressed in the early embryo, and subsequently becomes restricted to uncommitted or proliferative groups of cells as development proceeds. In the adult, expression persists in
30 the regenerating tissues of the ovaries and testes (reviewed in Fortini et al., 1993, Cell 75:1245-1247; Jan et al., 1993, Proc. Natl. Acad. Sci. USA 90:8305-8307; Sternberg, 1993,

Curr. Biol. 3:763-765; Greenwald, 1994, Curr. Opin. Genet. Dev. 4:556-562; Artavanis-Tsakonas et al., 1995, Science 268:225-232). Studies of the expression of Notch1, one of three known vertebrate homologs of Notch, in zebrafish and
5 *Xenopus*, have shown that the general patterns are similar; with Notch expression associated in general with non-terminally differentiated, proliferative cell populations. Tissues with high expression levels include the developing brain, eye and neural tube (Coffman et al., 1990, Science 249:1438-1441; Bierkamp et al., 1993, Mech. Dev. 43:87-100).
10 While studies in mammals have shown the expression of the corresponding Notch homologues to begin later in development, the proteins are expressed in dynamic patterns in tissues undergoing cell fate determination or rapid proliferation (Weinmaster et al., 1991, Development 113:199-205; Reaume et
15 al., 1992, Dev. Biol. 154:377-387; Stifani et al., 1992, Nature Genet. 2:119-127; Weinmaster et al., 1992, Development 116:931-941; Kopan et al., 1993, J. Cell Biol. 121:631-641; Lardelli et al., 1993, Exp. Cell Res. 204:364-372; Lardelli et al., 1994, Mech. Dev. 46:123-136; Henrique et al., 1995,
20 Nature 375:787-790; Horvitz et al., 1991, Nature 351:535-541; Franco del Amo et al., 1992, Development 115:737-744). Among the tissues in which mammalian Notch homologues are first expressed are the pre-somitic mesoderm and the developing neuroepithelium of the embryo. In the pre-somitic mesoderm,
25 expression of Notch1 is seen in all of the migrated mesoderm, and a particularly dense band is seen at the anterior edge of pre-somitic mesoderm. This expression has been shown to decrease once the somites have formed, indicating a role for Notch in the differentiation of somatic precursor cells
(Reaume et al., 1992, Dev. Biol. 154:377-387; Horvitz et al.,
30 1991, Nature 351:535-541). Similar expression patterns are seen for mouse Delta (Simske et al., 1995, Nature 375:142-145).

Within the developing mammalian nervous system, expression patterns of Notch homologue have been shown to be prominent in particular regions of the ventricular zone of the spinal cord, as well as in components of the peripheral nervous system, in an overlapping but non-identical pattern. Notch expression in the nervous system appears to be limited to regions of cellular proliferation, and is absent from nearby populations of recently differentiated cells (Weinmaster et al., 1991, Development 113:199-205; Reaume et al., 1992, Dev. Biol. 154:377-387; Weinmaster et al., 1992, Development 116:931-941; Kopan et al., 1993, J. Cell Biol. 121:631-641; Lardelli et al., 1993, Exp. Cell Res. 204:364-372; Lardelli et al., 1994, Mech. Dev. 46:123-136; Henrique et al., 1995, Nature 375:787-790; Horvitz et al., 1991, Nature 351:535-541). A rat Notch ligand is also expressed within the developing spinal cord, in distinct bands of the ventricular zone that overlap with the expression domains of the Notch genes. The spatio-temporal expression pattern of this ligand correlates well with the patterns of cells committing to spinal cord neuronal fates, which demonstrates the usefulness of Notch as a marker of populations of cells for neuronal fates (Henrique et al., 1995, Nature 375:787-790). This has also been suggested for vertebrate Delta homologues, whose expression domains also overlap with those of Notch1 (Larsson et al., 1994, Genomics 24:253-258; Fortini et al., 1993, Nature 365:555-557; Simske et al., 1995, Nature 375:142-145). In the cases of the *Xenopus* and chicken homologues, Delta is actually expressed only in scattered cells within the Notch1 expression domain, as would be expected from the lateral specification model, and these patterns "foreshadow" future patterns of neuronal differentiation (Larsson et al., 1994, Genomics 24:253-258; Fortini et al., 1993, Nature 365:555-557).

Other vertebrate studies of particular interest have focused on the expression of Notch homologues in developing sensory structures, including the retina, hair follicles and tooth buds. In the case of the *Xenopus* retina, 5 Notch1 is expressed in the undifferentiated cells of the central marginal zone and central retina (Coffman et al., 1990, Science 249:1439-1441; Mango et al., 1991, Nature 352:811-815). Studies in the rat have also demonstrated an association of Notch1 with differentiating cells in the developing retina have been interpreted to suggest that 10 Notch1 plays a role in successive cell fate choices in this tissue (Lyman et al., 1993, Proc. Natl. Acad. Sci. USA 90:10395-10399).

A detailed analysis of mouse Notch1 expression in the regenerating matrix cells of hair follicles was 15 undertaken to examine the potential participation of Notch proteins in epithelial/mesenchymal inductive interactions (Franco del Amo et al., 1992, Development 115:737-744). Such a role had originally been suggested for Notch1 based on the its expression in rat whiskers and tooth buds (Weinmaster et 20 al., 1991, Development 113:199-205). Notch1 expression was instead found to be limited to subsets of non-mitotic, differentiating cells that are not subject to epithelial/mesenchymal interactions, a finding that is consistent with Notch expression elsewhere.

Expression studies of Notch proteins in human 25 tissue and cell lines have also been reported. The aberrant expression of a truncated Notch1 RNA in human T-cell leukemia results from a translocation with a breakpoint in Notch1 (Ellisen et al., 1991, Cell 66:649-661). A study of human Notch1 expression during hematopoiesis has suggested a role 30 for Notch1 in the early differentiation of T-cell precursors (Mango et al., 1994, Development 120:2305-2315). Additional studies of human Notch1 and Notch2 expression have been

performed on adult tissue sections including both normal and neoplastic cervical and colon tissue. Notch1 and Notch2 appear to be expressed in overlapping patterns in differentiating populations of cells within squamous
5 epithelia of normal tissues that have been examined and are clearly not expressed in normal columnar epithelia, except in some of the precursor cells. Both proteins are expressed in neoplasias, in cases ranging from relatively benign squamous metaplasias to cancerous invasive adenocarcinomas in which columnar epithelia are replaced by these tumors (Mello et
10 al., 1994, Cell 77:95-106).

Insight into the developmental role and the general nature of Notch signaling has emerged from studies with truncated, constitutively activated forms of Notch in several species. These recombinantly engineered Notch forms, which
15 lack extracellular ligand-binding domains, resemble the naturally occurring oncogenic variants of mammalian Notch proteins and are constitutively activated using phenotypic criteria (Greenwald, 1994, Curr. Opin. Genet. Dev. 4:556; Fortini et al., 1993, Nature 365:555-557; Coffman et al.,
20 1993, Cell 73:659-671; Struhl et al., 1993, Cell 69:1073; Rebay et al., 1993, Genes Dev. 7:1949; Kopan et al., 1994, Development 120:2385; Roehl et al., 1993, Nature 364:632).

- Ubiquitous expression of activated Notch in the *Drosophila* embryo suppresses neuroblast segregation without impairing epidermal differentiation (Struhl et al., 1993,
25 Cell 69:331; Rebay et al., 1993, Genes Dev. 7:1949).

- Persistent expression of activated Notch in developing imaginal epithelia likewise results in an overproduction of epidermis at the expense of neural structures (Struhl et al., 1993, Cell 69:331).

30 - Neuroblast segregation occurs in temporal waves that are delayed but not prevented by transient expression of

activated Notch in the embryo (Struhl et al., 1993, Cell 69:331).

- Transient expression in well-defined cells of the *Drosophila* eye imaginal disc causes the cells to ignore their
5 normal inductive cues and to adopt alternative cell fates (Fortini et al., 1993, Nature 365:555-557).

- Studies utilizing transient expression of activated Notch in either the *Drosophila* embryo or the eye disc indicate that once Notch signaling activity has subsided,
10 cells may recover and differentiate properly or respond to later developmental cues (Fortini et al., 1993, Nature 365:555-557; Struhl et al., 1993, Cell 69:331).

For a general review on the Notch pathway and Notch signaling, see Artavanis-Tsakonas et al., 1995, Science 268:225-232.

15 Ligands, cytoplasmic effectors and nuclear elements of Notch signaling have been identified in *Drosophila*, and vertebrate counterparts have also been cloned (reviewed in Artavanis-Tsakonas et al., 1995, Science 268:225-232). While
20 protein interactions between the various elements have been documented, the biochemical nature of Notch signaling remains elusive. Expression of truncated forms of Notch reveal that Notch proteins without transmembrane and extracellular domains are translocated to the nucleus both in transgenic flies and in transfected mammalian or *Drosophila* cells
(Lieber et al., 1993, Genes and Development 7:1949-1965;
25 Fortini et al., 1993, Nature 365:555-557; Ahmad et al., 1995, Mechanisms of Development 53:78-85; Zagouras et al., 1995, Proc. Natl. Acad. Sci. USA 92:6414-6418). Sequence comparisons between mammalian and *Drosophila* Notch molecules, along with deletion analysis, have found two nuclear
30 localization sequences that reside on either side of the Ankyrin repeats (Stifani et al., 1992, Nature Genetics 2:119-127; Lieber et al., 1993, Genes and Development 7:1949-1965;

Kopan et al., 1994, Development 120:2385-2396). These findings prompted the speculation that Notch may be directly participating in nuclear events by means of a proteolytic cleavage and subsequent translocation of the intracellular fragment into the nucleus. However, conclusive functional evidence for such a hypothesis remains elusive (Artavanis-Tsakonas et al., 1995, Science 268:225-232).

Citation or identification of any reference in Section 2 or any other section of this application shall not be construed as an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The inventors have discovered that Delta is cleaved by the metalloprotease-disintegrin Kuzbanian (Kuz) into two fragments, a soluble amino-terminal fragment consisting essentially of the extracellular domain, and a membrane-bound fragment consisting essentially of the transmembrane domain and the intracellular domain. The soluble fragment of Delta, like the full length, membrane-bound Delta, is able to bind to Notch. Although not intending to be limited to any particular mechanism, Applicants believe that even though full length Delta is able to bind to Notch, it is the soluble fragment of Delta that is the actual ligand for Notch in vivo.

The detection or measurement of Delta activation, i.e., cleavage, is important in the study and manipulation of differentiation processes, since Delta plays a key role in cell fate (differentiation) determination, and since Delta is a ligand of Notch, Notch also playing a key role in cell fate (differentiation) determination. Molecules that modulate Delta and Notch function are important tools for studying and manipulating differentiation processes, e.g., in expanding cell populations without substantial differentiation

(International Publication WO 97/11716), in cancer studies and therapy (International Publication WO 94/07474), and differentiation studies on normal tissue. Molecules that allow the detection or measurement of Notch or Delta mRNA or protein levels or activity also have use in studying and manipulating differentiation processes. Accordingly, molecules that can be used to generate or detect anti-Delta antibodies or Delta nucleic acids have use in such detection or measurement.

One embodiment of the present invention is directed to a peptide of approximately 30 amino acids, and its encoding nucleic acids, of the toporythmic protein Delta that contains a sequence which is cleaved by the metalloprotease-disintegrin Kuzbanian (Kuz), (herein termed "cleavage peptide") as well as derivatives (e.g., fragments) and analogs thereof. For example, the Delta cleavage peptide consists of the sequence of amino acid Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), of amino acid Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), of amino acid Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), of amino acid Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and the sequence of amino acid Cys₅₆₄ to amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). Nucleic acids hybridizable to or complementary to the cleavage peptide encoding nucleic acids are also provided. In a specific embodiment, the Delta cleavage peptide is a portion of a mammalian Delta, preferably a human Delta. Such a peptide is believed to have the ability to modulate Kuz cleavage of Delta, and thus, Delta and Notch activation.

In a specific embodiment, the present invention is directed to a peptide comprising a fragment of a Delta protein, the amino acid sequence of the peptide consisting of the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse

Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). In another embodiment, a
5 fragment of a Delta protein of not more than 150 or 50 or 30 amino acids comprising a Delta sequence selected from the group consisting of amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in
10 chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and Cys₅₆₄ to amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). In yet another embodiment, the invention is directed to a peptide the amino acid sequence of which consists of amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to
15 amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

The invention is also directed to a derivative or
20 analog of the cleavage peptide which is functionally active, i.e., capable of displaying one or more known functional activities associated with the "wild type" cleavage peptide. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with the cleavage
25 peptide for binding) to an anti-Delta cleavage peptide antibody], immunogenicity (ability to generate antibody which binds to the cleavage peptide), ability to bind (or compete with the cleavage peptide for binding) to Kuz. The invention is further directed to a fragment (and derivatives or analogs
30 thereof) of the Delta cleavage peptide which is able to bind to Kuz.

Antibodies to the Delta cleavage peptide, its derivatives and analogs, are additionally provided.

Delta fragments that comprise the cleavage peptide sequence are also provided, as are fusion proteins comprising a Delta fragment containing a sequence of Delta that includes at least the cleavage peptide sequence, fused to a non-Delta sequence at the amino- and/or carboxy-terminal end of the Delta sequence. Concatamers of Delta fragments containing at least the cleavage peptide sequence (e.g., two, three, or more copies of a portion of the Delta sequence consisting of at least the cleavage peptide sequence) are also provided. In particular embodiments, the Delta fragments comprising the cleavage peptide sequence are not greater than 35, 50, 75, 100, 150, or 200 amino acids in length. In a specific embodiment, the present invention is directed to a chimeric protein comprising a Delta protein sequence fused to a non-Delta protein sequence, wherein the Delta protein sequence is a sequence of not more than 100 or 50 or 30 amino acids that comprises the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

In another embodiment, the present invention is directed to a peptide comprising an amino-terminal fragment of a full length Delta protein, which fragment is cleaved from the full length Delta protein by two proteolytic processing events, the cleavage of the signal peptide and the cleavage by Kuz, (herein termed "soluble Delta peptide" or "Dl^{EC}") as well as derivatives and analogs thereof. For example, the soluble Delta peptide amino acid sequence begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); begins at

amino acid Ser₂₄ and terminates between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or begins at
5 amino acid Ser₂₃ and terminates between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). Such a peptide is believed to have the ability to bind Notch, and thus modulate Delta and Notch activation.

The invention is also directed to a derivative or
10 analog of the soluble Delta peptide which is functionally active, i.e., capable of displaying one or more known functional activities associated with the "wild type" soluble peptide. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with the soluble peptide for binding) to an anti-Delta soluble peptide
15 antibody], immunogenicity (ability to generate antibody which binds to the soluble peptide), ability to bind (or compete with the soluble peptide for binding) to Notch.

Antibodies to the Delta soluble peptide, its derivatives and analogs, are additionally provided.

20 Methods of production of the Delta cleavage peptide, derivatives and analogs, e.g., by recombinant means, are also provided. Methods of production of the soluble Delta peptide, derivatives and analogs, e.g., by recombinant means, are also provided.

25 The present invention is also directed to certain compositions comprising and methods for production of protein complexes of Delta and Kuz. Specifically, in this embodiment, the invention is directed to complexes of Delta, and derivatives, fragments and analogs of Delta, with Kuz, and its derivatives, fragments and analogs (a complex of
30 Delta and Kuz is designated as "Delta:Kuz" herein). Methods of production of a Delta:Kuz complex, and a derivative or

analog thereof, e.g., by recombinant means, are also provided.

The present invention is also directed to certain compositions comprising and methods for production of protein
5 complexes of Notch and a soluble fragment of Delta consisting essentially of the extracellular domain that is liberated by the proteolytic processing of Delta by Kuz ("soluble Delta peptide" or "Dl^{EC}"). Specifically, in this embodiment, the invention is directed to complexes of the soluble Delta
10 peptide, and derivatives, fragments and analogs of the soluble Delta peptide, with Notch, and its derivatives, fragments and analogs (a complex of the soluble fragment of Delta and Notch is designated as "Dl^{EC}:Notch" herein). Methods of production of a Dl^{EC}:Notch complex, and a
15 derivative or analog thereof, e.g., by recombinant means, are also provided.

The invention is further directed to methods for modulating (i.e., inhibiting or enhancing) the activity of Notch or Delta or Kuz by contacting a cell expressing Notch or Delta or Kuz, or an organism comprising a cell expressing
20 Notch or Delta or Kuz, a peptide comprising a fragment of Delta having the amino acid sequence of about amino acid Cys₅₁₆ to about amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), of about amino acid Cys₅₁₅ to about amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), of about amino acid Cys₅₂₃ to
25 about amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), of about amino acid Cys₅₁₈ to about amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and the sequence of about amino acid Cys₅₆₄ to about amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). In specific embodiments, the peptide comprises 25,
30, 35, 40, 50, 100, 150, 200 or 250 amino acids of Delta.

30 The invention is further directed to methods for modulating (i.e., inhibiting or enhancing) the activity of Notch or Delta or Kuz or at least one of their signalling

pathways by contacting a cell or organism expressing Notch or Delta or Kuz with a peptide comprising a fragment of a Delta protein having the amino acid sequence beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); and the sequence beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

The invention is further directed to methods for modulating (*i.e.*, inhibiting or enhancing) the activity of a Delta:Kuz complex or a Dl^{EC}:Notch complex. The protein components of a Delta:Kuz complex or a Dl^{EC}:Notch complex have been implicated in cell fate and differentiation.

Accordingly, the present invention is directed to methods for screening a Delta:Kuz complex, as well as a derivative or analog of the complex, for the ability to alter cell fate or differentiation. The present invention is also directed to methods for screening a Dl^{EC}:Notch complex, as well as a derivative or analog of the complex, for the ability to alter cell fate or differentiation.

The present invention is also directed to therapeutic and diagnostic methods and compositions based on the Delta cleavage peptide and encoding nucleic acids, as well as on soluble Delta peptide and encoding nucleic acids. The invention provides for the treatment of disorders of cell fate and differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta cleavage

peptides and derivative and analogs (including fragments) thereof, antibodies thereto, nucleic acids encoding the Delta cleavage peptide, derivatives, or analogs, Delta cleavage peptide antisense nucleic acids, Delta:Kuz complexes and
5 antibodies thereto, and D1^{EC}:Notch complexes and antibodies thereto. In addition, such Therapeutics include soluble Delta peptides and derivatives and analogs thereof, antibodies thereto, nucleic acids encoding the soluble Delta peptides, derivatives, or analogs, and soluble Delta peptide antisense nucleic acids. In a preferred embodiment, a
10 Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a
15 nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch, Delta cleavage peptide and/or Kuz function (hereinafter "Antagonist Therapeutics") are administered for
20 therapeutic effect. In another embodiment, Therapeutics which promote Notch, Delta cleavage peptide and/or Kuz function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (e.g., cancer) or hypoproliferative
25 disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch, Delta cleavage peptide and or Kuz protein can be diagnosed by detecting such levels, as described more fully *infra*.

Yet another embodiment of the present invention is
30 directed to methods for detecting or measuring Delta activation by observing or measuring Delta cleavage products that are indicative of Delta activation. In one aspect of

this embodiment of the invention, the method for detecting or measuring Delta activation in a cell comprises detecting or measuring the expression of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM.

5 In yet another aspect, the method comprises detecting or measuring an amino-terminal fragment of full-length Delta terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9), between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10),

10 between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), or terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8). In yet another aspect, the method comprises detecting or measuring under reducing conditions, a soluble Delta

15 fragment of approximately 67 kilodaltons (D1^{EC}). In yet another aspect, the method comprises detecting or measuring a soluble Delta peptide having the amino acid sequence beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID

20 NO:10); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ

25 ID NO:8); and the sequence beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

The present invention is also directed to methods for detecting or measuring Kuz function by observing or

30 measuring Delta cleavage products that are indicative of Kuz function. In one aspect of this embodiment of the invention, the method for detecting or measuring Kuz function in a cell

comprises detecting or measuring the expression of one or more Delta cleavage products selected from the group consisting of $\Delta 1^{EC}$ and $\Delta 1^{TM}$. In yet another aspect, the method comprises detecting or measuring an amino-terminal fragment of full-length Delta which terminates between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta, between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta, between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta, between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta, or terminates between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta. In yet another aspect, the method comprises detecting or measuring under reducing conditions, a soluble Delta fragment of approximately 67 kilodaltons. In yet another aspect, the method comprises detecting or measuring a soluble Delta peptide having the amino acid sequence beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); and the sequence beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

In another embodiment, the present invention is also directed to methods for identifying a molecule that modulates Delta activation by detecting or measuring a change in the amount or pattern of Delta cleavage products. In one aspect of this embodiment of the invention, the method for identifying a modulator of Delta activation comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or

more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with the candidate
5 molecule indicates that the molecule modulates Delta activity.

In an alternative aspect, the method for identifying a modulator of Delta activation comprises contacting a candidate modulator molecule with a full length
10 Delta in the presence of a composition comprising Kuz and optionally other cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition and detecting or measuring the amount of Delta cleavage products
15 D1^{EC} and D1TM that result, in which a difference in the presence or amount of said Delta cleavage products compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

In yet another embodiment, the present invention is
20 also directed to methods for identifying a molecule that modulates Kuz function by detecting or measuring a change in the amount of Delta cleavage products that are necessary for Kuz function. In one aspect of this embodiment of the invention, the method for identifying a modulator of Kuz
25 function comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more
30 cleavage products compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Notch function.

In yet another embodiment, the present invention is also directed to methods for identifying a molecule that modulates Kuz function by detecting or measuring a change in the amount of Delta cleavage products that are indicative of Kuz function. In one aspect of this embodiment of the invention, the method for identifying a modulator of Kuz function comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected from the group consisting of Dl^{EC} and Dl^{TM} , in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

The present invention is also directed to therapeutic and prophylactic, as well as diagnostic, prognostic, and screening methods and compositions based upon the Delta:Kuz complex or the Dl^{EC} :Notch complex (and the nucleic acids encoding the individual proteins that participate in the complex). Therapeutic compounds of the invention include, but are not limited to, a Delta:Kuz complex, and a complex where one or both members of the complex is a derivative, fragment, homolog or analog of Delta or Kuz; antibodies to and nucleic acids encoding the foregoing; and antisense nucleic acids to the nucleotide sequences encoding the complex components. Diagnostic, prognostic and screening kits are also provided.

Animal models and methods of screening for modulators (i.e., agonists, and antagonists) of the activity of a Delta:Kuz complex or of a Dl^{EC} :Notch complex are also provided.

Methods of identifying molecules that inhibit, or alternatively, that increase formation of a Delta:Kuz complex or of a Dl^{EC} :Notch complex are also provided.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic diagram of the Notch signaling pathway. The Notch receptor can bind to either Delta or Serrate through its extracellular domain. Ligand binding can result in receptor multimerization that is stabilized by interactions between the intracellular ankyrin repeats of Notch and the cytoplasmic protein Deltex. These events can control the nuclear translocation of the DNA-binding protein Suppressor of Hairless and its known association with the Hairless protein. The transcriptional induction of the Enhancer of Split basic helix-loop-helix (bHLH) genes appears to depend on Notch signaling.

Figure 2 is a Notch homolog sequence comparison. The human Notch2 (humN2) (SEQ ID NO:1), human Notch1 (humN1) (SEQ ID NO:2), *Xenopus* Notch/Xotch (XenN) (SEQ ID NO:3), and *Drosophila* Notch (DrosN) (SEQ ID NO:4) protein sequences are aligned, with names indicated to the left and numbering to the right (Wharton et al., 1985, Cell 43:567-581; Coffman et al., 1990, Science 249:1438-1441; Ellisen et al., 1991, Cell 66:649-661; Stifani et al., 1992, Nature Genetics 2:119-127). Major Notch protein motifs are enclosed in boxes. Starting from the N-terminal, the boxed regions indicate: EGF repeats, Lin-12/Notch (LN) repeats, transmembrane domain (TM), Ankyrin repeats, and PEST-containing region. Also indicated are the putative CcN motif components (Stifani et al., 1992, Nature Genetics 2:119-127) nuclear localization signal (NLS, BNTS) and putative CKII and cdc2 phosphorylation sites. The calculated signal cleavage site is indicated with an arrow.

Figure 3 is a Delta homolog sequence comparison. The human Delta (HDL) (SEQ ID NO:5), mouse Delta (MDL) (SEQ ID NO:6), chick Delta (CDL) (SEQ ID NO:7), *Xenopus* Delta (XDL) (SEQ ID NO:8), and *Drosophila* Delta (DDL) (SEQ ID NO:9) protein sequences are aligned, with names indicated to the

left and numbering to the right. Major Delta protein motifs are labeled.

Figure 4A and 4B is the amino acid sequence (SEQ ID NO:10) and the nucleic acid sequence (SEQ ID NO:11),
5 respectfully, of human Delta.

Figure 5A and 5B is the amino acid sequence (SEQ ID NO:12) and the nucleic acid sequence (SEQ ID NO:13),
respectfully, of the human Kuz homolog.

Figures 6A-6F shows results of a genetic modifier screen that was carried out to identify genes that
10 genetically interact with *kuz*. A strain constitutively expressing a KuzDN construct in developing imaginal discs was used in the screen (expression of a KuzDN construct lacking the proprotein and metalloprotease domains was driven by a GAL4 line 32B) which causes adult mutant phenotypes,
15 including extra wing vein materials, mostly notably deltas at the ends of the longitudinal veins (denoted by arrowheads in Figure 6A), small and rough eyes, and extra bristles on the notum (denoted by arrowheads in Figure 6E). More than 2400 lethal P-element insertions were screened for phenotypic
20 modification effects on KuzDN. Seven P-insertions were found to cause significant reduction of the viability (semi-lethal) of the KuzDN flies when they are also heterozygous for each of the P-insertion. Preliminary characterization of these P-insertions revealed that two of them are Kuz alleles and one is a loss-of-function Delta allele while the nature of the
25 other insertions are unknown. Flies that carry an extra copy of the Delta gene (+/+ / +) with the KuzDN background (Figures 6B, 6F) show an almost complete suppression of the KuzDN phenotypes. (Figure 6C) An extra copy of Notch (+/+ / +) (Ramos et al., 1989, Genetics 123:337-348) alone has an
30 essentially normal phenotype (Figure 6C). Notch (+/+ / +) gives negligible suppression of the KuzDN phenotype in KuzDN flies (Figure 6D).

Figures 7A-7E show that a soluble fragment of Delta (Dl^{EC}) is released constitutively in S2 cells *in vivo*. Figure 7A: Expression of Delta (Dl) antigen in stably transfected S2 cells (Rebay, et al., 1991, Cell 67:687-699) is detected by SDS-PAGE and western blotting with monoclonal antibody 9B of reduced (+Bme) and non-reduced (-Bme) cell extracts (c) and culture media (m). A product consistent with full length Delta is clearly detectable in the cell extract (MW~ 83,000 Daltons non-reduced and 90,000 Daltons reduced). A significant amount of a product of greater mobility is seen in the media (MW~ 62,000 Daltons non-reduced and 67,000 Daltons reduced) that is consistent in size with the extracellular domain of Delta (estimated MW~ 65,000 Daltons) and is referred to as Dl^{EC}. A 40-fold higher affinity of the antibody was observed for the non-reduced versus reduced Delta and was compensated for by increased protein load (4X) and exposure times (10X) in the reduced samples. Figure 7B: Bands of the same mobility are seen in extracts of wild type *Drosophila* embryos (16hr). Note that 1, 3, 5 and 10 embryos loaded on the gel demonstrate that the antigen is barely detectable in a single embryo ("1") but becomes clearer with the greater number of embryos loaded ("10"). Figure 7C: Affinity purified Dl^{EC} migrates at MW~ 62,000 Daltons under reducing conditions and at MW~ 67,000 Daltons under non-reducing conditions on a coomassie blue-stained SDS-PAGE gel. Figure 7D: Schematic of the *Drosophila* Delta protein demonstrates the DSL domain (DSL), the epidermal growth factor like repeats (EGF) and the transmembrane domain (TM). Amino acid numbering of N-terminus, the beginning of the TM domain and the C-terminus is shown. Figure 7E: Thirteen cycles of N-terminal amino acid sequence analysis of Dl^{EC} is shown with alignment to the *Drosophila* (dDl), *Xenopus* (xDl) and human (hDl) Delta amino acid sequences. The arrow indicates the conserved serine residue in the position of the

N-terminus of Dl^{EC} and the potential site of signal peptide processing for Dl .

Figures 8A-8D shows that Kuz plays a direct role in Delta processing *in vivo* and *in vitro*. Figure 8A: The first two panels (-): Expression of Delta and Dl^{EC} are apparent by western blotting with the 9B antibody in the cell pellet (c) and the medium (m) in S2 cells transiently transfected with full length Delta (Fehon, et al., 1990, Cell 61:523-534). The second two panels (Kuz): Cotransfection of S2 cells with Kuz and Delta results in an increase in the Dl^{EC} fragment in the cell culture media (m) which correlates with an apparent decrease in Delta in the cell pellet (c). The third two panels (KuzDN): Cotransfection with dominant negative Kuz dramatically decreases the Dl^{EC} observed in the media (m) and corresponds with greater amounts of full length Delta in the cell pellet (c). Figure 8B: Cotransfection of Kuz and KuzDN with Notch was done under identical experimental conditions as for Delta and western blotted with the 9C6 Notch intracellular domain antibody (Fehon, et al., 1990, Cell 61:523-534) demonstrates a negligible effect on the processing of Notch as seen by the invariant levels of N^{TM} , the constitutively processed form of Notch (Blaumueller et al., 1997, Cell 90:281-291). Figure 8C: The metalloprotease inhibitors EDTA and 1,10-phenanthroline inhibit the endogenous S2 cell proteolytic activity yielding Dl^{EC} . The left panel demonstrates the accumulation of Dl^{EC} at various time points up to 60 minutes in the medium of S2 cells stably expressing full length Delta (Rebay, et al., 1991, Cell 67:687-699). The right panel shows the accumulation of Dl^{EC} at 60 minutes in the presence of EDTA (5, 10, 15 mM) and 1,10-phenanthroline (5, 10 mM). Relatively high concentrations of the chelators were required to overcome the concentrations Ca^{2+} (~8.6 mM) and other metal ions in the media and serum. Higher concentrations of 1,10-

phenanthroline proved to alter cell morphology. Both of these reagents, which are well documented metalloprotease inhibitors, inhibit accumulation of Dl^{EC} in the media. Figure 8D: Delta processing is inhibited in Kuz -/- embryos. Nine
5 Kuz +/- and Kuz -/- embryos were identified by morphology and the extracts analyzed by SDS-PAGE and western blotting with antibody 9B. Dl^{EC} is absent in Kuz -/- embryos and demonstrates a higher level of full length Delta compared to Kuz +/- embryos.

10 Figures 9A-9C shows that Dl^{EC} binds to Notch, competes for Notch-Delta interaction and acts as an agonist of the Notch pathway. Figure 9A: The Dl^{EC} fragment specifically binds to Notch expressing S2 cells and does not bind to S2 cells alone. Notch expressing S2 cells (lane 1,
2) incubated in the absence (lane 1) or presence (lane 2) of
15 Dl^{EC} (lane 6) were sedimented through a sucrose cushion and the extract was western blotted with antibody 9B. Dl^{EC} was prepared as a 5X concentrate of 16 hour culture media (Sang's M3) of 0.7mM $CuSO_4$ induced Delta-S2 cells. Notch-S2 and nontransfected S2 cells were induced with 0.7 mM $CuSO_4$ for
20 16hrs in media with 5% serum. The cells were collected by centrifugation and washed once in serum free media with 1% bovine serum albumin (BSA) and resuspended at 2×10^6 cells/mL in M3, 1% BSA. 250 μ L of cells were added to 100 μ L of Dl^{EC} concentrate, raised to 500 μ L with M3, 1% BSA and incubated one hour at room temperature on a rocking table at five
25 oscillations per minute. The mixture was layered over a cushion of 20% sucrose, 20mM TRIS-HCl, 150mM NaCl, 2mM $CaCl_2$, 1% BSA, pH 7.4, in microfuge tubes that had previously been blocked with 1% BSA. The tubes were centrifuged at 14,000 rpm for 3 minutes and the supernatant aspirated. The cell
30 pellets were washed two times with cold serum free media without resuspension of the pellet. The pellet was then lysed and dissolved in SDS-PAGE sample buffer without β -

mercaptoethanol and boiled for five minutes. The proteins were resolved by SDS-PAGE and western blotting with the 9B antibody. Lane 3 and 4 show parallel incubations with S2 cells in the absence (lane 3) or presence (lane 4) of Dl^{EC} .

5 Figure 9B: Preincubation of Notch-S2 cells with Dl^{EC} concentrate reduces their subsequent rate of aggregation with Delta-S2 cells as measured turbidimetrically with transmitted light at 320nm. At the concentration shown (1X Dl^{EC} , closed circles), a 60% inhibition in the initial rate of aggregation was seen compared to control media concentrate (1X ΔECN , closed squares). The error bars show the standard deviation of the mean of triplicate determinations. Figure 9C shows the effect of Dl^{EC} on primary cultured cortical neurons in the representative images as labeled: (I) seven to ten days in vitro cortical neurons before treatment, (II) cultured in the presence of ΔECN media, (III) cultured in the presence of Dl^{EC} media, (IV) affinity purified Dl^{EC} , and (V) buffer control for purified Dl^{EC} . The graph represents the mean length of neurites per neuron. Each bar represents the mean \pm SEM of three separate experimental trials. Primary cortical neurons exhibit multipolar morphology and the extensive neurite network in control cultures (I), cultures in the presence of ΔECN media (II) and buffer control of purified Dl^{EC} (V). Note the decrease in the mean neurite length per neuron and limited neurite branching in cultures treated with Dl^{EC} media (III) and purified Dl^{EC} (IV). Scale bar = 50 μm .

25 Figure 10 is a schematic diagram comparing the soluble fragment of Delta (Dl^{EC}) that is clipped by Kuz with DLS.

Figure 11 shows the amino acid sequence of the Delta cleavage peptide of *Drosophila* Delta (SEQ ID NO:9).
30 Bold arrows indicate potential cleavage sites identified by data from both C-terminal sequence analysis and LC/MS; dashed arrows indicate potential cleavage sites identified by only

one of the analyses. (A) indicates the alanine instead of the threonine reported by Vässin et al., 1987, EMBO J. 6:3431-3440.

5 5. DETAILED DESCRIPTION OF THE INVENTION

 The inventors have discovered that Delta is cleaved by the metalloprotease-disintegrin Kuzbanian (Kuz) into two fragments, a soluble amino-terminal fragment consisting essentially of the extracellular domain, and a membrane-bound fragment consisting essentially of the transmembrane domain and the intracellular domain. The soluble fragment of Delta, like the full length, membrane-bound Delta, is able to bind to Notch. Although not intending to be limited to any particular mechanism, Applicants believe that even though full length Delta is able to bind to Notch, it is the soluble fragment of Delta that is the actual ligand for Notch in vivo.

 The detection or measurement of Delta activation, i.e., cleavage, is important in the study and manipulation of differentiation processes, since Delta plays a key role in cell fate (differentiation) determination, and since Delta is a ligand of Notch, Notch also playing a key role in cell fate (differentiation) determination. Molecules that modulate Delta and Notch function are important tools for studying and manipulating differentiation processes, e.g., in expanding cell populations without substantial differentiation (International Publication WO 97/11716), in cancer studies and therapy (International Publication WO 94/07474), and differentiation studies on normal tissue. Molecules that allow the detection or measurement of Notch or Delta mRNA or protein levels or activity also have use in studying and manipulating differentiation processes. Accordingly, molecules that can be used to generate or detect anti-Delta

antibodies or Delta nucleic acids have use in such detection or measurement.

One embodiment of the present invention is directed to a peptide of approximately 30 amino acids, and its
5 encoding nucleic acids, of the toporythmic protein Delta that contains a sequence which is cleaved by the metalloprotease-disintegrin Kuzbanian (Kuz), (herein termed "cleavage peptide") as well as derivatives (e.g., fragments) and analogs thereof. For example, the Delta cleavage peptide consists of the sequence of amino acid Cys₅₁₆ to amino acid
10 Phe₅₄₃ in human Delta, of amino acid Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta, of amino acid Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta, of amino acid Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta, and the sequence of amino acid Cys₅₆₄ to amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta. Nucleic acids
15 hybridizable to or complementary to the cleavage peptide encoding nucleic acids are also provided. In a specific embodiment, the Delta cleavage peptide is a portion of a mammalian Delta, preferably a human Delta. Such a peptide is believed to have the ability to modulate Kuz cleavage of
20 Delta, and thus, Delta and Notch activation.

The invention is also directed to a derivative or analog of the cleavage peptide which is functionally active, i.e., capable of displaying one or more known functional activities associated with the "wild type" cleavage peptide. Such functional activities include but are not limited to
25 antigenicity [ability to bind (or compete with the cleavage peptide for binding) to an anti-Delta cleavage peptide antibody], immunogenicity (ability to generate antibody which binds to the cleavage peptide), ability to bind (or compete with the cleavage peptide for binding) to Kuz. The invention
30 is further directed to a fragment (and derivatives or analogs thereof) of the Delta cleavage peptide which is able to bind to Kuz.

Antibodies to the Delta cleavage peptide, its derivatives and analogs, are additionally provided.

Delta fragments that comprise the cleavage peptide sequence are also provided, as are fusion proteins comprising
5 a Delta fragment containing a sequence of Delta that includes at least the cleavage peptide sequence, fused to a non-Delta sequence at the amino- and/or carboxy-terminal end of the Delta sequence. Concatamers of Delta fragments containing at least the cleavage peptide sequence (e.g., two, three, or
10 more copies of a portion of the Delta sequence consisting of at least the cleavage peptide sequence) are also provided. In particular embodiments, the Delta fragments comprising the cleavage peptide sequence are not greater than 35, 50, 75, 100, 150, or 200 amino acids in length.

Methods of production of the Delta cleavage
15 peptide, derivatives and analogs, e.g., by recombinant means, are also provided.

In another embodiment, the present invention is directed to a peptide comprising an amino-terminal fragment of a full length Delta protein, which fragment is cleaved
20 from the full length Delta protein by two proteolytic processing events, the cleavage of the signal peptide and the cleavage by Kuz (herein termed "soluble Delta peptide" or "Dl^{EC}") as well as derivatives and analogs thereof. For example, the soluble Delta peptide amino acid sequence begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₆
25 and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); begins at amino acid Ser₂₄ and terminates between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), begins at
30 amino acid Ser₂₂ and terminates between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or begins at amino acid Ser₂₃ and terminates between amino acid Cys₅₆₄ and

amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). Such a peptide is believed to have the ability to bind Notch, and thus modulate Delta and Notch activation.

The invention is also directed to a derivative or
5 analog of the soluble Delta peptide which is functionally active, i.e., capable of displaying one or more known functional activities associated with the "wild type" soluble peptide. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with the
10 soluble peptide for binding) to an anti-Delta soluble peptide antibody], immunogenicity (ability to generate antibody which binds to the soluble peptide), ability to bind (or compete with the soluble peptide for binding) to Notch.

Antibodies to the Delta soluble peptide, its derivatives and analogs, are additionally provided.

15 Methods of production of the soluble Delta peptide, derivatives and analogs, e.g., by recombinant means, are also provided.

The present invention is also directed to certain compositions comprising and methods for production of protein
20 complexes of Delta and Kuz. Specifically, in this embodiment, the invention is directed to complexes of Delta, and derivatives, fragments and analogs of Delta, with Kuz, and its derivatives, fragments and analogs (a complex of Delta and Kuz is designated as "Delta:Kuz" herein). Methods
25 of production of a Delta:Kuz complex, and a derivative or analog thereof, e.g., by recombinant means, are also provided.

The present invention is also directed to certain compositions and methods for production of protein complexes with Notch of the soluble fragment of Delta liberated by Kuz.
30 Specifically, in this embodiment, the invention is directed to complexes of the soluble Delta peptide, and derivatives, fragments and analogs of the soluble fragment, with Notch,

and its derivatives, fragments and analogs (a complex of the soluble fragment of Delta and Notch is designated as "Dl^{EC}:Notch" herein). Methods of production of a Dl^{EC}:Notch complex, and a derivative or analog thereof, e.g., by
5 recombinant means, are also provided.

The invention is further directed to methods for modulating (i.e., inhibiting or enhancing) the activity of Notch or Delta or Kuz by contacting a cell expressing Notch or Delta or Kuz, or an organism comprising a cell expressing
10 Notch or Delta or Kuz, a peptide comprising a fragment of Delta having the amino acid sequence of about amino acid Cys₅₁₆ to about amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), of about amino acid Cys₅₁₅ to about amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), of about amino acid Cys₅₂₃ to about amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), of about
15 amino acid Cys₅₁₈ to about amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and the sequence of about amino acid Cys₅₆₄ to about amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). In specific embodiments, the peptide comprises 25, 30, 35, 40, 50, 100, 150, 200 or 250 amino acids of Delta.

20 The invention is further directed to methods for modulating (i.e., inhibiting or enhancing) the activity of Notch or Delta or Kuz or at least one of their signalling pathways by contacting a cell or organism expressing Notch or Delta or Kuz with a peptide comprising a fragment of a Delta
25 protein having the amino acid sequence beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino
30 acid Phe₅₅₁ in chick Delta (SEQ ID NO:7); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); and the sequence

beginning at amino acid Ser₂₃, and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

The invention is further directed to methods for
5 modulating (i.e., inhibiting or enhancing) the activity of a Delta:Kuz complex or the activity of a D1^{EC}:Notch complex. The protein components of a Delta:Kuz complex and of a D1^{EC}:Notch complex have been implicated in cell fate and differentiation. Accordingly, the present invention is directed to methods for screening a Delta:Kuz complex, as
10 well as a derivative or analog of the complex, for the ability to alter cell fate or differentiation. The present invention is also directed to methods for screening a D1^{EC}:Notch complex, as well as a derivative or analog of the complex, for the ability to alter cell fate or
15 differentiation.

The present invention is also directed to therapeutic and diagnostic methods and compositions based on the Delta cleavage peptide and encoding nucleic acids, as well as on soluble Delta peptides and encoding nucleic acids.
20 The invention provides for the treatment of disorders of cell fate and differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta cleavage peptides and derivative and analogs (including fragments) thereof, antibodies thereto, nucleic acids encoding the Delta
25 cleavage peptide, derivatives, or analogs, Delta cleavage peptide antisense nucleic acids, Delta:Kuz complexes and antibodies thereto, and D1^{EC}:Notch complexes and antibodies thereto. In addition, such Therapeutics include soluble Delta peptides and derivatives and analogs thereof,
30 antibodies thereto, nucleic acids encoding the soluble Delta peptides, derivatives, or analogs, and soluble Delta peptide antisense nucleic acids. In a preferred embodiment, a

Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a

5 Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch, Delta cleavage peptide and/or Kuz function (hereinafter "Antagonist Therapeutics") are administered for
10 therapeutic effect. In another embodiment, Therapeutics which promote Notch, Delta cleavage peptide and/or Kuz function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular
15 hyperproliferative (e.g., cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch, Delta cleavage peptide and or Kuz protein can be diagnosed by detecting such levels, as described more fully *infra*.

20 Yet another embodiment of the present invention is directed to methods for detecting or measuring Delta activation by observing or measuring Delta cleavage products that are indicative of Delta activation. In one aspect of this embodiment of the invention, the method for detecting or
25 measuring Delta activation in a cell comprises detecting or measuring the expression of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM. In yet another aspect, the method comprises detecting or measuring an amino-terminal fragment of full-length Delta terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or
30 Gln₅₉₄ in *Drosophila* Delta, between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta, between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta, between amino acid Cys₅₂₃ and amino

acid Phe₅₅₁ in chick Delta, or terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta. In yet another aspect, the method comprises detecting or measuring under reducing conditions, a soluble Delta fragment of
5 approximately 67 kilodaltons. In yet another aspect, the method comprises detecting or measuring a soluble Delta peptide having the amino acid sequence beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino
10 acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); and the sequence
15 beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

The present invention is also directed to methods for detecting or measuring Kuz function by observing or
20 measuring Delta cleavage products that are indicative of Kuz function. In one aspect of this embodiment of the invention, the method for detecting or measuring Kuz function in a cell comprises detecting or measuring the expression of one or more Delta cleavage products selected from the group consisting of Dl^{EC} and DlTM. In yet another aspect, the method
25 comprises detecting or measuring an amino-terminal fragment of full-length Delta which terminates between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta, between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta, between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta,
30 between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta, or terminates between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta. In yet another aspect, the method

comprises detecting or measuring under reducing conditions, a soluble Delta fragment of approximately 67 kilodaltons. In yet another aspect, the method comprises detecting or measuring a soluble Delta peptide having the amino acid sequence beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); and the sequence beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃, or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

In another embodiment, the present invention is also directed to methods for identifying a molecule that modulates Delta activation by detecting or measuring a change in the amount or pattern of Delta cleavage products. In one aspect of this embodiment of the invention, the method for identifying a modulator of Delta activation comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

In an alternative aspect, the method for identifying a modulator of Delta activation comprises contacting a candidate modulator molecule with a full length Delta in the presence of a composition comprising Kuz and optionally other cellular proteins, under conditions

conductive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition and detecting or measuring the amount of Delta cleavage products D1^{EC} and D1TM that result, in which a difference in the
5 presence or amount of said Delta cleavage products compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

In yet another embodiment, the present invention is also directed to methods for identifying a molecule that
10 modulates Notch function by detecting or measuring a change in the amount of Delta cleavage products that are necessary for Notch function. In one aspect of this embodiment of the invention, the method for identifying a modulator of Notch function comprises providing a cell with a candidate
15 modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with
20 the candidate molecule indicates that the molecule modulates Notch function.

In yet another embodiment, the present invention is also directed to methods for identifying a molecule that modulates Kuz function by detecting or measuring a change in the amount of Delta cleavage products that are indicative of
25 Kuz function. In one aspect of this embodiment of the invention, the method for identifying a modulator of Kuz function comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected
30 from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with

the candidate molecule indicates that the molecule modulates Kuz function.

The present invention is also directed to therapeutic and prophylactic, as well as diagnostic, prognostic, and screening methods and compositions based upon the Delta:Kuz complex or a Dl^{EC} :Notch complex (and the nucleic acids encoding the individual proteins that participate in the complex). Therapeutic compounds of the invention include, but are not limited to, a Delta:Kuz complex, and a complex where one or both members of the complex is a derivative, fragment, homolog or analog of Delta or Kuz; antibodies to and nucleic acids encoding the foregoing; and antisense nucleic acids to the nucleotide sequences encoding the complex components. Diagnostic, prognostic and screening kits are also provided.

Animal models and methods of screening for modulators (*i.e.*, agonists, and antagonists) of the activity of a Delta:Kuz complex or the activity of a Dl^{EC} :Notch complex are also provided.

Methods of identifying molecules that inhibit, or alternatively, that increase formation of a Delta:Kuz complex or of a Dl^{EC} :Notch complex are also provided.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections that follow.

5.1 DELTA CLEAVAGE PEPTIDES, SOLUBLE DELTA PEPTIDES AND DELTA:KUZ PROTEIN COMPLEXES

5.1.1 DELTA CLEAVAGE PEPTIDES AND SOLUBLE DELTA PEPTIDES

Delta encoding nucleic acids from both vertebrate and non-vertebrate species have been cloned, see *e.g.*, International Patent Publication WO 97/01571 for a description of vertebrate, including human, Delta encoding nucleic acids. Human Delta encoding sequences and the

encoded amino acid sequence is available in GenBank under Accession No. AF003522 and are depicted in Figures 4A and 4B. The nucleotide sequence coding for a Delta cleavage peptide, or for a soluble Delta peptide, or a functionally active
5 fragment or other derivative thereof, can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be
10 supplied by the native Delta gene and/or its flanking regions. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such
15 as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation
20 elements may be used. In a specific embodiment, the human Delta cleavage peptide is expressed. In another specific embodiment, the human soluble Delta peptide is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to
25 construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a Delta cleavage
30 peptide or peptide fragment thereof may be regulated by a second nucleic acid sequence so that the Delta cleavage peptide is expressed in a host transformed with the

recombinant DNA molecule. For example, expression of a Delta cleavage peptide may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control Delta cleavage peptide expression include, but are
5 not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the
10 metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also
15 "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl.
20 Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following
25 animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409;
30 MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region

which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in
5 testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-
10 antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region
15 which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the
20 hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Expression vectors containing inserts of nucleic acids encoding a Delta cleavage peptide or encoding a soluble Delta peptide can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of
25 "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to the inserted Delta cleavage peptide coding sequences. In the second approach, the recombinant
30 vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics,

transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if the Delta cleavage peptide encoding nucleic acids are inserted within the marker gene
5 sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the encoded cleavage
10 peptide in *in vitro* assay systems, e.g., binding to Kuz, binding with antibody.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and
15 growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as
20 vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or
25 modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered Delta cleavage peptide may be controlled. Furthermore, different host cells
30 have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, cleavage [e.g., of signal

sequence)) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous mammalian Delta cleavage peptide, or to ensure "native" glycosylation of a heterologous mammalian soluble Delta peptide. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

In other specific embodiments, the Delta cleavage peptide, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the peptide, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

One embodiment of the present invention is directed to a peptide of approximately 30 amino acids, and its encoding nucleic acids, of the toporythmic protein Delta that contains a sequence which is cleaved by the metalloprotease-disintegrin Kuzbanian (Kuz), (herein termed "cleavage peptide") as well as derivatives (e.g., fragments) and analogs thereof. For example, the Delta cleavage peptide consists of the sequence of about amino acid Cys₅₁₆ to about amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), of about

amino acid Cys₅₁₅ to about amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), of about amino acid Cys₅₂₃ to about amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), of about amino acid Cys₅₁₈ to about amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and
5 the sequence of about amino acid Cys₅₆₄ to about amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). Such a peptide is believed to have the ability to modulate Kuz cleavage of Delta, and thus, Delta and Notch activation. In a specific embodiment, the Delta cleavage peptide is a
10 portion of a mammalian Delta, preferably a human Delta.

The invention further relates to Delta cleavage peptides, and derivatives (including but not limited to fragments) and analogs of Delta cleavage peptides. Nucleic acids encoding Delta cleavage peptide derivatives and peptide
15 analogs are also provided. In particular aspects, the peptides, derivatives, or analogs are of mouse, chicken, frog, rat, pig, cow, dog, monkey, or human Delta cleavage peptides.

The production and use of derivatives and analogs related to Delta cleavage peptides are within the scope of
20 the present invention. In a specific embodiment, the derivative or analog is functionally active, i.e., capable of exhibiting one or more functional activities associated with wild-type Delta cleavage peptide. As one example, such derivatives or analogs which have the desired immunogenicity or antigenicity can be used, for example, in immunoassays,
25 for immunization, for inhibition of Delta activity, etc. Such molecules which retain, or alternatively inhibit, a desired Delta property, e.g., binding to kuz or other toporythmic proteins, can be used as inducers, or inhibitors, respectively, of such property and its physiological
30 correlates. Derivatives or analogs of a Delta cleavage peptide can be tested for the desired activity by procedures

known in the art, including but not limited to the assays described herein.

In particular, Delta cleavage peptide derivatives can be made by altering Delta cleavage peptide encoding sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a Delta cleavage peptide may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of the encoding Delta cleavage peptide genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the Delta cleavage peptide derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a Delta protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The

negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a specific embodiment, fragments of Delta that comprise the cleavage peptide sequence are also provided. In particular embodiments, the Delta fragments comprising the cleavage peptide are not greater than 35, 50, 75, 100, 150, or 200 amino acids in length. For example, a Delta fragment containing the cleavage peptide sequence comprises the cleavage peptide sequence and 35 contiguous amino-terminal amino acids. In another example, the fragment comprises the cleavage peptide sequence and 100 contiguous amino-terminal amino acids. In yet another example, the fragment comprises the cleavage peptide sequence and 50 contiguous carboxy-terminal amino acids. In yet another example, the fragment comprises the cleavage peptide sequence and 50 contiguous amino-terminal amino acids and 50 contiguous carboxy-terminal amino acids. In yet another embodiment, oncatamers of Delta fragments containing at least the cleavage peptide sequence (e.g., two, three, or more copies of a portion of the Delta sequence consisting of at least the cleavage peptide sequence) are also provided.

The Delta cleavage peptide derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned Delta gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated in vitro. In the production of the gene encoding a derivative or analog of a Delta cleavage peptide, care should be taken to ensure that

the modified gene remains within the same translational reading frame as Delta, uninterrupted by translational stop signals.

Additionally, the Delta cleavage peptide-encoding
5 nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art
10 can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem 253:6551), etc. PCR primers containing sequence changes can be used in PCR to introduce such changes into the amplified fragments.

15 Manipulations of the Delta cleavage peptide sequence may also be made at the protein level. Included within the scope of the invention are Delta cleavage peptide fragments or other derivatives or analogs which are differentially modified during or after translation, e.g., by
20 glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by
25 cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives of Delta cleavage peptide can be chemically synthesized. Furthermore,
30 if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the Delta sequence. Non-classical amino acids include but

are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, 5 designer amino acids such as β -methyl amino acids, α -methyl amino acids, and $N\alpha$ -methyl amino acids and amino acid analogs in general.

In a specific embodiment, the Delta cleavage peptide derivative is a chimeric, or fusion, peptide comprising a Delta cleavage peptide or fragment thereof 10 joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a Delta cleavage peptide-coding sequence joined 15 in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by 20 methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. In a specific embodiment, a chimeric nucleic acid encoding a Delta cleavage peptide with a heterologous signal sequence is expressed such that the chimeric protein is expressed extracellularly by the 25 cell.

The invention is also directed to a derivative or analog of the cleavage peptide which is functionally active, i.e., capable of displaying one or more known functional activities associated with the "wild type" cleavage peptide. 30 Such functional activities include but are not limited to antigenicity [ability to bind (or compete with the cleavage peptide for binding) to an anti-Delta cleavage peptide

antibody], immunogenicity (ability to generate antibody which binds to the cleavage peptide), ability to bind (or compete with the cleavage peptide for binding) to Kuz. The invention is further directed to a fragment (and derivatives or analogs thereof) of the Delta cleavage peptide which is able to bind to Kuz.

In another embodiment, the present invention is directed to a peptide comprising an amino-terminal fragment of a full length Delta protein, which fragment is cleaved from the full length Delta protein by two proteolytic processing events, the cleavage of the signal peptide and the cleavage by Kuz, (herein termed "soluble Delta peptide") as well as derivatives and analogs thereof. For example, the soluble Delta peptide amino acid sequence begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); begins at amino acid Ser₂₄ and terminates between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or begins at amino acid Ser₂₃ and terminates between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). Such a peptide is believed to have the ability to bind Notch, and thus modulate Delta and Notch activation.

The invention further relates to soluble Delta peptides, and derivatives (including but not limited to fragments) and analogs of soluble Delta peptides. Nucleic acids encoding soluble Delta peptide derivatives and peptide analogs are also provided. In particular aspects, the peptides, derivatives, or analogs are of mouse, chicken, frog, rat, pig, cow, dog, monkey, or human soluble Delta peptides.

The production and use of derivatives and analogs related to soluble Delta peptides are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, i.e., capable of exhibiting one or more functional activities associated with wild-type soluble Delta peptide. As one example, such derivatives or analogs which have the desired immunogenicity or antigenicity can be used, for example, in immunoassays, for immunization, for promotion of Delta activity, etc. Such molecules which retain, or alternatively inhibit, a desired Delta property, e.g., binding to Notch or other toporythmic proteins, can be used as inducers, or inhibitors, respectively, of such property and its physiological correlates. Derivatives or analogs of a soluble Delta peptide can be tested for the desired activity by procedures known in the art, including but not limited to the assays described herein.

In particular, soluble Delta peptide derivatives can be made by altering soluble Delta peptide encoding sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a soluble Delta peptide may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of the encoding soluble Delta peptide genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the soluble Delta peptide derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a Delta protein including altered sequences in which functionally equivalent amino acid residues are substituted

for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

The soluble Delta peptide derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned Delta gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of a soluble Delta peptide, care should be taken to ensure that the modified gene remains within the same translational reading frame as Delta, uninterrupted by translational stop signals.

Additionally, the soluble Delta peptide-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or

termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further in vitro modification. Any technique for mutagenesis known in the art
5 can be used, including but not limited to, in vitro site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem 253:6551), etc. PCR primers containing sequence changes can be used in PCR to introduce such changes into the amplified fragments.

10 Manipulations of the soluble Delta peptide sequence may also be made at the protein level. Included within the scope of the invention are soluble Delta peptide fragments or other derivatives or analogs which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by
15 known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin,
20 chymotrypsin, papain, V8 protease, NaBH_4 ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc. In a specific embodiment, N- or C-terminal modifications are made, e.g., N-acetylation.

In addition, analogs and derivatives of soluble
25 Delta peptide can be chemically synthesized. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the Delta sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid, hydroxyproline,
30 sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, designer amino acids such as β -methyl amino acids, $\text{C}\alpha$ -methyl

amino acids, and N α -methyl amino acids and amino acid analogs in general.

In a specific embodiment, the soluble Delta peptide derivative is a chimeric, or fusion, peptide comprising a soluble Delta peptide or fragment thereof joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a soluble Delta peptide-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. In a specific embodiment, a chimeric nucleic acid encoding a soluble Delta peptide with a heterologous signal sequence is expressed such that the chimeric protein is expressed extracellularly by the cell.

The invention is also directed to a derivative or analog of the soluble peptide which is functionally active, i.e., capable of displaying one or more known functional activities associated with the "wild type" soluble peptide. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with the soluble peptide for binding) to an anti-soluble Delta peptide antibody], immunogenicity (ability to generate antibody which binds to the soluble peptide), ability to bind (or compete with the soluble peptide for binding) to Notch. The invention is further directed to a fragment (and derivatives or analogs thereof) of the soluble Delta peptide which is able to bind to Notch.

5.1.2 PROTEIN COMPLEXES OF DELTA AND KUZ AND DELTA AND NOTCH

The present invention is directed to a Delta:Kuz protein complex. The present invention is also directed to a Dl^{EC}:Notch protein complex. Delta, Kuz and Notch have been cloned, see e.g., WO 92/19734, WO 97/01571 and WO 98/08933. Figure 2 depicts the amino acid sequences of several Notch homologs (SEQ ID NOS:1, 2, 3 and 4), including human Notch (SEQ ID NOS:1 and 2). Figure 3 depicts the amino acid sequences of several Delta homologs (SEQ ID NOS:5, 6, 7, 8 and 9) and the nucleic acid sequence encoding human Delta is depicted in Figure 4B (SEQ ID NO:13). The amino acid sequence (SEQ ID NO:12) of the human homolog of Kuz and its encoding nucleic acid sequence (SEQ ID NO:13) is depicted in Figures 5A and 5B, respectively. Dl^{EC} is the amino-terminal fragment of full length Delta consisting of essentially the extracellular domain of wild-type Delta that is liberated when Kuz cleaves Delta. The Dl^{EC} fragment is soluble and begins at amino acid Ser₂₃ and terminates between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9), begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), begins at amino acid Ser₂₄ and terminates between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), or begins at amino acid Ser₂₂ and terminates between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8).

In a preferred embodiment of the present invention, the Delta:Kuz complex or the Dl^{EC}:Notch complex is a complex of human proteins. The invention is also directed to complexes of derivatives (including fragments) and analogs of Delta with Kuz, complexes of Delta with derivatives

(including fragments) and analogs of Kuz, and complexes of derivatives (including fragments) and analogs of Delta and Kuz (as used herein, fragment, derivative, homolog or analog of a Delta:Kuz complex includes complexes where one or both members of the complex are fragments, derivatives or analogs of the wild-type Delta or Kuz protein). The present invention is also directed to complexes of derivatives (including fragments) and analogs of D1^{EC} with Notch, complexes of D1^{EC} with derivatives (including fragments) and analogs of Notch, and complexes of derivatives (including fragments) and analogs of D1^{EC} and Notch (as used herein, fragment, derivative, homolog or analog of a D1^{EC}:Notch complex includes complexes where one or both members of the complex are fragments, derivatives or analogs of the wild-type D1^{EC} or Notch protein). In a preferred embodiment, the D1^{EC}:Notch complex in which one or both members of the complex is a fragment, derivative, homolog or analog of the wild type protein is a functionally-active D1^{EC}:Notch complex. In particular aspects, the native proteins, or derivatives or analogs of Delta, Notch and/or Kuz are obtained from an animal, e.g., mouse, rat, pig, cow, dog, monkey, human, fly, frog. In another aspect, the native proteins are obtained from plants.

As used herein, a "functionally active Delta:Kuz complex" refers to that material displaying one or more known functional attributes of a complex of wild type Delta with wild type Kuz, including protein-protein binding, binding to a Delta-, a Kuz-, and/or a Delta:Kuz complex-specific antibody, or has the functional attribute(s) of Delta, Kuz, and/or a Delta:Kuz complex involved in cell fate and differentiation.

As used herein, a "functionally active D1^{EC}:Notch complex" refers to that material displaying one or more known functional attributes of a complex of wild type D1^{EC} with wild

type Notch, including protein-protein binding, binding to a Dl^{EC} -, a Notch-, and/or a Dl^{EC} :Notch complex-specific antibody, or has the functional attribute(s) of Dl^{EC} , Notch, and/or a Dl^{EC} :Notch complex involved in cell fate and differentiation.

5 The present invention is also directed to a method of screening a Delta:Kuz complex, particularly a complex of Delta with Kuz for the ability to alter a cell function, particularly those cell functions in which Delta and/or Kuz has been implicated, including, e.g., physiological processes such as cell fate determination and differentiation, binding
10 to an anti-Delta:Kuz complex antibody, etc., and other activities as they are described in the art. The present invention is also directed to a method of screening a Dl^{EC} :Notch complex, particularly a complex of Dl^{EC} with Notch for the ability to alter a cell function, particularly those
15 cell functions in which Dl^{EC} and/or Notch has been implicated, including, e.g., physiological processes such as cell fate determination and differentiation, binding to an anti- Dl^{EC} :Notch complex antibody, etc., and other activities as they are described in the art.

20 The present invention is also directed to a method for screening a complex of a derivative, fragment, or analog of Delta and/or Kuz for the ability to alter a cell function such as differentiation. For example, such derivatives or analogs which have the desired immunogenicity or antigenicity can be used in immunoassays, for immunization, for inhibition
25 of Delta:Kuz complex activity, etc. Derivatives or analogs that retain, or alternatively lack or inhibit, a property of interest (e.g., participation in a Delta:Kuz complex) can be used as an inducer, or inhibitor, respectively, of such a property and its physiological correlate. The present
30 invention is also directed to a method for screening a complex of a derivative, fragment, or analog of Dl^{EC} and/or Notch for the ability to alter a cell function such as

differentiation. For example, such derivatives or analogs which have the desired immunogenicity or antigenicity can be used in immunoassays, for immunization, for inhibition of Dl^{EC} :Notch complex activity, etc. Derivatives or analogs that
5 retain, or alternatively lack or inhibit, a property of interest (e.g., participation in a Dl^{EC} :Notch complex) can be used as an inducer, or inhibitor, respectively, of such a property and its physiological correlate.

A specific embodiment of the present invention is directed to a Delta:Kuz complex of a fragment of Delta and/or
10 a fragment of Kuz that can be bound by an anti-Delta antibody and/or bound by an anti-Kuz antibody, respectively, or bound by an antibody specific for a Delta:Kuz complex. Another specific embodiment of the present invention is directed to a Dl^{EC} :Notch complex of a fragment of Dl^{EC} and/or a fragment of
15 Notch that can be bound by an anti- Dl^{EC} antibody and/or bound by an anti-Notch antibody, respectively, or bound by an antibody specific for a Dl^{EC} :Notch complex.

Fragments and other derivatives or analogs of a Delta:Kuz complex or of a Dl^{EC} :Notch complex can be tested for
20 the desired activity by procedures known in the art, including but not limited to the assays described *infra*.

In specific embodiments, the present invention is directed to a Delta:Kuz complex or to a Dl^{EC} :Notch complex comprising a fragment of one or both members of the complex.
25 In a preferred embodiment, these fragments consist of, but are not exclusive to fragments of Kuz, identified as interacting with Delta in a modified yeast matrix mating assay or genetic screen. Fragments, or proteins comprising fragments, lacking a region of either member of the complex, are also provided. Nucleic acids encoding the foregoing are
30 provided in the present invention.

Nucleic acids encoding Delta, Notch and Kuz are known, and in addition can be obtained by any method known in

the art, e.g., by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of each sequence, and/or by cloning from a cDNA or genomic library using an oligonucleotide specific for each nucleotide sequence.

5 Homologs (e.g., nucleic acids encoding Delta, Notch and Kuz of species other than human) or other related sequences (e.g., paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe, using methods well known in the art for nucleic acid hybridization and cloning.

10 The encoded human Delta, Kuz and Notch proteins, which are depicted in Figures 4A, 5A and 2, respectively (SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NOS:1 and 2, respectively) either alone or in a complex, can be obtained by methods well known in the art for protein purification and recombinant
15 protein expression. For recombinant expression of one or more of the proteins, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein can be inserted into an appropriate expression vector, i.e., a vector that contains the necessary elements for the
20 transcription and translation of the inserted protein coding sequence. The necessary transcriptional and translational signals can also be supplied by the native promoter of the Delta, Kuz and Notch genes, and/or their flanking regions.

A variety of host-vector systems may be utilized to
25 express the protein coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The
30 expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized,

any one of a number of suitable transcription and translation elements may be used.

In a preferred embodiment, a Delta:Kuz complex is obtained by expressing the entire Delta coding sequence and
5 the entire Kuz coding sequence in the same cell, either under the control of the same promoter or two separate promoters. In yet another embodiment, a derivative, fragment or homolog of Delta and/or a derivative, fragment or homolog of Kuz are recombinantly expressed. Preferably the derivative, fragment
10 or homolog of Delta and/or the Kuz protein forms a complex with a binding partner identified by a binding assay, and more preferably forms a complex that binds to an anti-Delta:Kuz complex antibody. In another preferred embodiment, a SDelta:Notch complex is obtained by expressing the entire
15 Dl^{EC} coding sequence and the entire Notch coding sequence in the same cell, either under the control of the same promoter or two separate promoters. In yet another embodiment, a derivative, fragment or homolog of Dl^{EC} and/or a derivative, fragment or homolog of Notch are recombinantly expressed. Preferably the derivative, fragment or homolog of Dl^{EC} and/or
20 the Notch protein forms a complex with a binding partner identified by a binding assay, and more preferably forms a complex that binds to an anti-Dl^{EC}:Notch complex antibody.

Any method available in the art can be used for the insertion of DNA fragments into a vector to construct
25 expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinant techniques (genetic recombination). Expression
30 of nucleic acid sequences encoding Delta, Kuz and Notch, or a derivative, fragment or homolog thereof, may be regulated by a second nucleic acid sequence so that the gene or fragment thereof is expressed in a host transformed with the

recombinant DNA molecule(s). For example, expression of the proteins may be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the genes for Delta, Notch or Kuz. Promoters that
5 may be used include but are not limited to those described in Section 5.1.1.

In a specific embodiment, a vector is used that comprises a promoter operably linked to nucleic acid sequences encoding Delta, Notch and/or Kuz, or a fragment, derivative or homolog thereof, one or more origins of
10 replication, and optionally, one or more selectable markers (e.g., an antibiotic resistance gene). In a preferred embodiment, a vector is used that comprises a promoter operably linked to nucleic acid sequences encoding both Delta and Kuz, or both D1^{EC} and Notch, one or more origins of
15 replication, and optionally, one or more selectable markers.

In another specific embodiment, an expression vector containing the coding sequence, or a portion thereof, of Delta and Kuz, or of D1^{EC} and Notch, either together or separately, is made by subcloning the gene sequences into the
20 EcoRI restriction site of each of the three pGEX vectors (glutathione S-transferase expression vectors; Smith and Johnson, 1988, Gene 7:31-40). This allows for the expression of products in the correct reading frame.

Expression vectors containing the sequences of interest can be identified by three general approaches: (a)
25 nucleic acid hybridization, (b) presence or absence of "marker" gene function, and (c) expression of the inserted sequences. In the first approach, Delta, Notch and Kuz sequences can be detected by nucleic acid hybridization to probes comprising sequences homologous and complementary to
30 the inserted sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker"

functions (e.g., resistance to antibiotics, occlusion body formation in baculovirus, etc.) caused by insertion of the sequences of interest in the vector. For example, if a Delta or Kuz gene, or portion thereof, is inserted within the
5 marker gene sequence of the vector, recombinants containing the Delta or Kuz fragment will be identified by the absence of the marker gene function (e.g., loss of beta-galactosidase activity). In the third approach, recombinant expression vectors can be identified by assaying for the Delta and Kuz
10 expressed by the recombinant vector. Such assays can be based, for example, on the physical or functional properties of the interacting species in *in vitro* assay systems, e.g., formation of a Delta:Kuz complex or binding to an anti-Delta, anti-Kuz, or anti-Delta:Kuz complex antibody.

Once recombinant Delta, Notch and Kuz molecules are
15 identified and the complexes or individual proteins isolated, several methods known in the art can be used to propagate them. Using a suitable host system and growth conditions, recombinant expression vectors can be propagated and amplified in quantity. As previously described, the
20 expression vectors or derivatives which can be used include, but are not limited to, human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus, yeast vectors; bacteriophage vectors such as lambda phage; and plasmid and cosmid vectors.

In addition, a host cell strain may be chosen that
25 modulates the expression of the inserted sequences, or modifies or processes the expressed proteins in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically-engineered Delta, Notch and/or Kuz may be
30 controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g.,

glycosylation, phosphorylation, etc.) of proteins.

Appropriate cell lines or host systems can be chosen to ensure that the desired modification and processing of the foreign protein is achieved. For example, expression in a bacterial system can be used to produce an unglycosylated core protein, while expression in mammalian cells ensures "native" glycosylation of a heterologous protein. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

10 In other specific embodiments, the Delta, Notch and/or Kuz protein or a fragment, homolog or derivative thereof, may be expressed as fusion or chimeric protein products comprising the protein, fragment, homolog, or derivative joined via a peptide bond to a heterologous protein sequence of a different protein. Such chimeric products can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acids to each other by methods known in the art, in the proper coding frame, and expressing the chimeric products in a suitable host by methods commonly known in the art. Alternatively, such a chimeric product can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Chimeric genes comprising portions of Delta, Notch and/or Kuz fused to any heterologous protein-encoding sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of Delta, Notch and/or Kuz of at least six amino acids.

In a specific embodiment, fusion proteins are provided that contain the interacting domains of the Delta protein and Kuz, or the interacting domains of D1^{EC} and Notch, and, optionally, a peptide linker between the two domains, where such a linker promotes the interaction of the Delta and Kuz binding domains or promotes the interaction of the D1^{EC} and Notch binding domains. These fusion proteins may be

particularly useful where the stability of the interaction is desirable (due to the formation of the complex as an intra-molecular reaction), for example, in production of antibodies specific to the Delta:Kuz complex or specific to the

5 D1^{EC}:Notch complex.

In particular, Delta, Notch and/or Kuz derivatives can be made by altering their sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences that encode

10 substantially the same amino acid sequence as a Delta, Notch or Kuz gene or cDNA can be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of the Delta, Notch or Kuz genes that are altered by the substitution of

15 different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the Delta, Notch or Kuz derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino

20 acid sequence of Delta, Notch or Kuz, including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by

25 another amino acid of a similar polarity that acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline,

30 phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged

(basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a specific embodiment of the invention, the
5 nucleic acids encoding proteins and proteins consisting of or comprising a fragment of Delta, Notch or Kuz consisting of at least 6 (continuous) amino acids of Delta, Notch or Kuz are provided. In other embodiments, the fragment consists of at least 10, 20, 30, 40, or 50 amino acids of Delta and Kuz or
10 D1^{EC} and Notch. In specific embodiments, such fragments are not larger than 35, 100 or 200 amino acids. Derivatives or analogs of Delta, Notch and Kuz include, but are not limited, to molecules comprising regions that are substantially homologous to Delta, Notch or Kuz, in various embodiments, by at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% identity
15 over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to a sequence encoding Delta, Notch or Kuz under stringent,
20 moderately stringent, or nonstringent conditions.

The Delta, Notch and Kuz derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned
25 Delta, Notch and Kuz gene sequences can be modified by any of numerous strategies known in the art (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The sequences can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic
30 modification if desired, isolated, and ligated in vitro. In the production of the gene encoding a derivative, homolog or analog of Delta, Notch or Kuz, care should be taken to ensure

that the modified gene retains the original translational reading frame, uninterrupted by translational stop signals, in the gene region where the desired activity is encoded.

Additionally, the Delta-, Notch- and/or Kuz-
5 encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art
10 can be used, including but not limited to, chemical mutagenesis and *in vitro* site-directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem 253:6551-6558), amplification with PCR primers containing a mutation, etc.

Once a recombinant cell expressing Delta, Notch
15 and/or Kuz, or fragment or derivative thereof, is identified, the individual gene product or complex can be isolated and analyzed. This is achieved by assays based on the physical and/or functional properties of the protein or complex, including, but not limited to, radioactive labeling of the
20 product followed by analysis by gel electrophoresis, immunoassay, cross-linking to marker-labeled product, etc.

The Delta:Kuz or D1^{EC}:Notch complexes may be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the complexes or proteins), including but not
25 restricted to column chromatography (e.g., ion exchange, affinity, gel exclusion, reversed-phase high pressure, fast protein liquid, etc.), differential centrifugation, differential solubility, or by any other standard technique used for the purification of proteins. Functional properties
30 may be evaluated using any suitable assay known in the art.

Alternatively, once Delta or its derivative, or Kuz or its derivative, or Notch or its derivative, is identified,

the amino acid sequence of the protein can be deduced from the nucleic acid sequence of the chimeric gene from which it was encoded. As a result, the protein or its derivative can be synthesized by standard chemical methods known in the art
5 (e.g., Hunkapiller et al., 1984, Nature 310: 105-111).

In a specific embodiment of the present invention, such Delta:Kuz complexes, whether produced by recombinant DNA techniques, chemical synthesis methods, or by purification from native sources include, but are not limited to, those containing, as a primary amino acid sequence, all or part of
10 the amino acid sequences substantially as depicted in Figures 3 and 5A-5B (SEQ ID NOS:5, 6, 7, 8 and 9 and SEQ ID NO:12, respectively), as well as fragments and other analogs and derivatives thereof, including proteins homologous thereto. In another specific embodiment of the present invention, such
15 D1^{EC}:Notch complexes, whether produced by recombinant DNA techniques, chemical synthesis methods, or by purification from native sources include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequences substantially as depicted in Figures
20 2 and 3 (SEQ ID NOS:5, 6, 7, 8 and 9 and SEQ ID NOS:1, 2, 3, and 4, respectively), as well as fragments and other analogs and derivatives thereof, including proteins homologous thereto.

Manipulations of Delta, Notch and/or Kuz sequences may be made at the protein level. Included within the scope
25 of one embodiment of the invention is a complex of a Delta fragment or a Kuz fragment and Delta or Kuz fragments, derivatives and analogs that are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by
30 known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried

out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH_4 , acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

In specific embodiments, the Delta, Notch and/or Kuz amino acid sequences are modified to include a fluorescent label. In another specific embodiment, Delta, Notch and/or Kuz are modified to have a heterofunctional reagent; such heterofunctional reagents can be used to crosslink the members of the complex.

In addition, complexes of analogs and derivatives of Delta and/or Kuz, or Dl^{EC} and/or Notch, can be chemically synthesized. For example, a peptide corresponding to a portion of Delta and/or Kuz, which comprises the desired domain or mediates the desired activity *in vitro* (e.g., Delta:Kuz complex formation) can be synthesized by use of a peptide synthesizer. Furthermore, if desired, non-classical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the Delta and/or Kuz.

Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid (4-Abu), 2-aminobutyric acid (2- Abu), 6-amino hexanoic acid (Ahx), 2-amino isobutyric acid (2-Aib), 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, $\text{C}\alpha$ -methyl amino acids, $\text{N}\alpha$ -methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

In cases where natural products are suspected of being mutant or are isolated from new species, the amino acid

sequence of Delta, Notch or Kuz isolated from the natural source, as well as those expressed *in vitro*, or from synthesized expression vectors *in vivo* or *in vitro*, can be determined from analysis of the DNA sequence, or
5 alternatively, by direct sequencing of the isolated protein. Such analysis can be performed by manual sequencing or through use of an automated amino acid sequenator.

The Delta:Kuz or D1^{EC}:Notch complexes can also be analyzed by hydrophilicity analysis (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828). A hydrophilicity
10 profile can be used to identify the hydrophobic and hydrophilic regions of the proteins, and help predict their orientation in designing substrates for experimental manipulation, such as in binding experiments, antibody synthesis, etc. Secondary structural analysis can also be
15 done to identify regions of Delta, Notch and/or Kuz, or their derivatives, that assume specific structures (Chou and Fasman, 1974, Biochemistry 13:222-23). Manipulation, translation, secondary structure prediction, hydrophilicity and hydrophobicity profile predictions, open reading frame
20 prediction and plotting, and determination of sequence homologies, etc., can be accomplished using computer software programs available in the art.

Other methods of structural analysis including but not limited to X-ray crystallography (Engstrom, 1974 Biochem. Exp. Biol. 11:7-13), mass spectroscopy and gas chromatography
25 (Methods in Protein Science, J. Wiley and Sons, New York, 1997), and computer modeling (Fletterick and Zoller, eds., 1986, Computer Graphics and Molecular Modeling, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, New York) can also be
30 employed.

5.2 ANTIBODIES

According to one embodiment of the present invention, a Delta cleavage peptide, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which recognize such an immunogen. According to another embodiment of the present invention, a soluble Delta peptide, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which recognize such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to human Delta cleavage peptide are produced. In another specific embodiment, antibodies to human soluble Delta peptide are produced.

According to another embodiment of the present invention, the Delta:Kuz complex or a fragment, derivative or homolog thereof, or the D1^{EC}:Notch complex or a fragment, derivative or homolog thereof, may be used as an immunogen to generate antibodies which immunospecifically bind such immunogen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to a complex of human Delta and human Kuz are produced. In another specific embodiment, antibodies to a complex of human D1^{EC} and human Notch are produced. In another embodiment, a complex formed from a fragment of Delta and a fragment of Kuz, which fragments contain the protein domain that interacts with the other member of the complex, are used as an immunogen for antibody production.

Various procedures known in the art may be used for the production of polyclonal antibodies to a Delta cleavage peptide or derivative or analog, or to a soluble Delta peptide or derivative or analog, or to a protein complex of

the present invention. For the production of antibody, various host animals can be immunized by injection with the native Delta cleavage peptide, or D1^{EC} or Notch or Kuz, or a synthetic version, or derivative (e.g., fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

For preparation of monoclonal antibodies directed toward, for example, a Delta cleavage peptide sequence or analog thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies"

(Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for, e.g.,
5 Delta cleavage peptide together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce, for example, Delta
10 cleavage peptide-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments
15 with the desired specificity for Delta proteins, derivatives, or analogs.

Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the
20 F(ab')₂ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

25 In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize, for example, a Delta cleavage peptide, one may assay generated hybridomas
30 for a product which binds to a Delta cleavage peptide. For selection of an antibody immunospecific to human Delta cleavage peptide, one can select on the basis of positive

binding to human Delta cleavage peptide and a lack of binding to *Drosophila* Delta cleavage peptide.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the protein sequences of the invention, e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

In another embodiment of the invention (see *infra*), anti-Delta cleavage peptide antibodies specific for the Delta cleavage peptide and fragments thereof containing the binding domain are Therapeutics. In yet another embodiment of the invention, an anti-Delta:Kuz complex antibody or a fragment thereof containing the binding domain, is a Therapeutic. In yet another embodiment of the invention, an anti-soluble Delta peptide antibody or a fragment thereof containing the binding domain, is a Therapeutic.

5.3 DETECTION OF THE ACTIVE FORM OF DELTA

The present invention is directed to methods for detecting or measuring Delta activation by observing or measuring Delta cleavage products that are indicative of Delta activation. In one aspect of this embodiment of the invention, the method for detecting or measuring Delta activation in a cell comprises detecting or measuring the expression of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM. In yet another aspect, the method comprises detecting or measuring an amino-terminal fragment of full-length Delta beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID

NO:6), beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), or beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in Xenopus Delta (SEQ ID NO:8). In yet another aspect, the method comprises detecting or measuring under reducing conditions, a soluble Delta fragment of approximately 67 kilodaltons.

In another embodiment, the present invention is also directed to methods for identifying a molecule that modulates Delta activation by detecting or measuring a change in the amount or pattern of Delta cleavage products. In one aspect of this embodiment of the invention, the method for identifying a modulator of Notch activation comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Notch cleavage products selected from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

In an alternative aspect, the method for identifying a modulator of Delta activation comprises contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition and detecting or measuring the amount of Delta cleavage products D1^{EC} and D1TM that result, in which a difference in the presence or amount of said Notch cleavage products compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

Any method known in the art for detecting or measuring the expression of Delta cleavage products indicative of Delta activation can be used. For example, and not by way of limitation, one such method of detection of the active form of Delta by detecting one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, or by detecting an amino-terminal fragment of full-length Delta beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), or beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8). In yet another aspect, the method comprises detecting or measuring under reducing conditions, a soluble Delta fragment of approximately 67 kilodaltons.

Detection of such cleavage products can be done, e.g., by immunoprecipitating the cleavage products with an anti-Delta antibody or binding to anti-Delta antibody on an immunoaffinity column or immobilized on a plate or in a well, or visualizing the fragments by Western blotting. In a specific embodiment, the cleavage products can be labelled by general cell surface labeling, or, alternatively, by pulse labeling the cells by incubation in culture medium containing a radioactive label, or, alternatively, it can be anti-Delta antibody (or antibody binding partner) that is labeled rather than the Delta cleavage products.

Another method to detect the active form of Delta is to use a Delta ligand or binding fragment thereof, such as

Notch, to bind to Delta (e.g., when the ligand is labeled), or to recover Delta by coimmunoprecipitating with the appropriate anti-Delta ligand antibody to co-immunoprecipitate Delta cleavage products, etc.

5 Similar procedures to those described *supra* can be used to make antibodies to domains of other proteins (particularly toporythmic proteins) that bind or otherwise interact with Delta (e.g., binding fragments of Notch).

10 The cell in which Delta activation is detected or measured can be any cell, e.g., one that endogenously or recombinantly expresses Delta. The cell can be vertebrate, insect (e.g., *Drosophila*), *C. elegans*, mammalian, bovine, murine, rat, avian, fish, primate, human, etc. The Delta which is expressed can be vertebrate, insect, *C. elegans*, mammalian, bovine, murine, rat, avian, fish, primate, human,
15 etc. The cell can be a cell of primary tissue, a cell line, or of an animal containing and expressing a Delta transgene. For example, the transgenic animal can be a *Drosophila* (e.g., *melanogaster*) or a *C. elegans*. In a preferred embodiment, the transgene encodes a human Delta. Transgenic animals can
20 be made by standard methods well known in the art (e.g., by use of P element transposons as a vector in *Drosophila*).

5.4 METHODS OF IDENTIFYING MODULATORS OF DELTA ACTIVATION

25 In one embodiment of the invention, methods are provided for the identification of modulators, e.g., inhibitors, antagonists, or agonists, of Delta activation by detecting the ability of the modulators to effect cleavage of full length Delta. In one aspect of this embodiment of the invention, the method for identifying a modulator of Delta
30 activation comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected

from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates
5 Delta activity. In yet another aspect, the method comprises providing a cell with a candidate modulator molecule and detecting or measuring the amount of the expression by the cell of an amino-terminal fragment of full-length Delta beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta
10 (SEQ ID NO:9), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), beginning at amino acid Ser₂₄ and terminating
15 between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), or beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); in which a difference in the presence or amount of said fragment compared to a Delta cell
20 not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

In yet another aspect, the method comprises providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of a
25 soluble Delta fragment of approximately 67 kilodaltons, in which a difference in the presence or amount of said soluble fragment compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

In yet another aspect of this embodiment of the
30 invention, the method for identifying a modulator of Delta activation comprises contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and

optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition, and detecting or measuring the amount of Delta cleavage products DI^{EC} and/or DI^{TM} that result, in which a difference in the presence or amount of said Delta cleavage product(s) compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Delta activity. In another aspect, the method for identifying a modulator of Delta activation comprises contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition, and detecting or measuring an amino-terminal fragment of full-length Delta beginning at amino acid Ser_{23} and terminating between amino acid Cys_{564} and amino acid Ala_{593} or Gln_{594} in *Drosophila* Delta (SEQ ID NO:9), beginning at amino acid Ser_{22} and terminating between amino acid Cys_{516} and amino acid Phe_{543} in human Delta (SEQ ID NO:10), beginning at amino acid Ser_{22} and terminating between amino acid Cys_{515} and amino acid Phe_{543} in mouse Delta (SEQ ID NO:6), beginning at amino acid Ser_{24} and terminating between amino acid Cys_{523} and amino acid Phe_{551} in chick Delta (SEQ ID NO:7), or beginning at amino acid Ser_{22} and terminating between amino acid Cys_{518} and amino acid Phe_{544} in *Xenopus* Delta (SEQ ID NO:8), in which a difference in the presence or amount of said fragment compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

In yet another aspect, the method for identifying a modulator of Delta activation comprises contacting a candidate modulator molecule with a full length Delta in the

presence of Kuz and optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition and detecting or measuring the amount of a soluble Delta fragment of approximately 67 kilodaltons, in which a difference in the presence or amount of said soluble fragment compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

10 In a specific aspect of the embodiment using a composition comprising cellular proteins, the composition comprising cellular proteins is a cell lysate made from cells which recombinantly express Delta. In another specific aspect of this embodiment, the composition comprising cellular proteins is a cell lysate made from cells which endogenously express Delta.

Detection or measurement of Delta cleavage products can be carried out by methods well known in the art and/or those methods disclosed in Section 5.1, *supra*.

20 The cells used in the methods of this embodiment can either endogenously or recombinantly express Delta. Examples of the cell types and Delta protein that can be expressed are described in Section 5.1. Recombinant Delta expression is carried out by introducing Delta encoding nucleic acids into expression vectors and subsequently introducing the vectors into a cell to express Delta or simply introducing Delta encoding nucleic acids into a cell for expression. Nucleic acids encoding vertebrate and non-vertebrate Delta have been cloned and sequenced and their expression is well known in the art. See, for example, International Publication WO 97/01571, which is incorporated by reference in their entirety herein. Expression can be from expression vectors or intrachromosomal.

Any method known to those of skill in the art for the insertion of Delta-DNA into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a Delta protein may be regulated by a second nucleic acid sequence so that the Delta protein is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a Delta protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control Delta gene expression include, but are not limited to, those described in Section 5.1.

In the methods of the invention in which full-length Delta is incubated with compositions comprising cellular proteins (e.g., cell lysates or cell fractions) in the presence of candidate cleavage (and thus Delta activation) modulators the expression of Delta should be such that full length Delta is expressed and proteolytic cleavage of Delta is kept to a minimum such that Delta cleavage products are easily detected over any background proteolysis. There are several methods known in the art to keep proteolysis to a minimum. For example, one manner to keep Delta cleavage to a minimum is to express Delta in cells concurrently with Brefeldin A treatment. Another manner is to express Delta in cells which do not contain Kuz or to express Delta in an *in vitro* transcription-translation system in the presence of a protease inhibitor such as phenylmethylsulfonylfluoride (PMSF).

5.5 METHODS OF IDENTIFYING MODULATORS OF KUZ ACTIVATION

In one embodiment of the invention, methods are provided for the identification of modulators, e.g., inhibitors, antagonists, or agonists, of Kuz function by detecting the ability of the modulators to effect cleavage of full length Delta. In one aspect of this embodiment of the invention, the method for identifying a modulator of Kuz function comprises providing a Delta expressing cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more cleavage products compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Kuz function. In yet another aspect, the method comprises providing a Delta expressing cell with a candidate modulator molecule and detecting or measuring the amount of the expression by the cell of an amino-terminal fragment of full-length Delta beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), or beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); in which a difference in the presence or amount of said fragment compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

In yet another aspect, the method comprises providing a Delta expressing cell with a candidate modulator molecule and detecting or measuring the expression by the cell of a soluble Delta fragment of approximately 67
5 kilodaltons, in which a difference in the presence or amount of said soluble fragment compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

In yet another aspect of this embodiment of the invention, the method for identifying a modulator of Kuz
10 function comprises contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition,
15 and detecting or measuring the amount of Delta cleavage products D1^{EC} and/or D1TM that result, in which a difference in the presence or amount of said Delta cleavage product(s) compared to a full-length Delta in presence of said composition not contacted with the candidate molecule
20 indicates that the molecule modulates Kuz activity. In another aspect, the method for identifying a modulator of Kuz function comprises contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by
25 Kuz and optionally one or more components of the composition, and detecting or measuring an amino-terminal fragment of full-length Delta beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9), beginning at amino
30 acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino

acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), or beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), in which a difference in the presence or amount of said fragment compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

10 In yet another aspect, the method for identifying a modulator of Kuz function comprises contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of
15 the composition and detecting or measuring the amount of a soluble Delta fragment of approximately 67 kilodaltons, in which a difference in the presence or amount of said soluble fragment compared to a full-length Delta in presence of said composition not contacted with the candidate molecule
20 indicates that the molecule modulates Kuz function.

In a specific aspect of the embodiment using a composition comprising cellular proteins, the composition comprising cellular proteins is a cell lysate made from cells which recombinantly express Kuz. In another specific aspect of this embodiment, the composition comprising cellular
25 proteins is a cell lysate made from cells which endogenously express Kuz.

Detection or measurement of Delta cleavage products can be carried out by methods well known in the art and/or those methods disclosed in Section 5.1, *supra*.

30 The cells used in the methods of this embodiment can either endogenously or recombinantly express Kuz. Examples of the cell types and Kuz protein that can be

expressed are described in Section 5.1. Recombinant Kuz expression is carried out by introducing Kuz encoding nucleic acids into expression vectors and subsequently introducing the vectors into a cell to express Kuz or simply introducing Kuz encoding nucleic acids into a cell for expression.
5 Nucleic acids encoding vertebrate and non-vertebrate Kuz have been cloned and sequenced and their expression is well known in the art. See, for example, International Publication WO 98/08933, which is incorporated by reference in its entirety herein. Expression can be from expression vectors or
10 intrachromosomal.

Any method known to those of skill in the art for the insertion of Kuz-DNA into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational
15 control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a Delta or Kuz protein may be regulated by a second nucleic acid sequence so
20 that the Kuz protein is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a Kuz protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control Delta gene expression include, but are not limited to, those described in Section 5.1.

25 In the methods of the invention in which full-length Delta and Kuz is incubated with compositions comprising cellular proteins (e.g., cell lysates or cell fractions) in the presence of candidate cleavage (and thus Delta activation) modulators the expression of Delta should
30 be such that full length Delta is expressed and proteolytic cleavage of Delta is kept to a minimum such that Delta cleavage products are easily detected over any background

proteolysis. There are several methods known in the art to keep proteolysis to a minimum. For example, one manner to keep Delta cleavage to a minimum is to express Delta in cells concurrently with Brefeldin A treatment. Another manner is to express Kuz in cells which do not contain Delta or to express Kuz in an in vitro transcription-translation system in the presence of a protease inhibitor such as phenylmethylsulfonylfluoride (PMSF).

10 5.6 **METHODS OF IDENTIFYING MODULATORS OF DELTA:KUZ
COMPLEX OR D1^{EC}:NOTCH COMPLEX ACTIVITY**

Delta:Kuz or D1^{EC}:Notch complexes, and derivatives, fragments and analogs thereof, nucleic acids encoding Delta, Notch and Kuz as well as derivatives, fragments and analogs of the nucleic acids, can be used to screen for compounds that bind to, or modulate the function of a Delta:Kuz complex or a D1^{EC}:Notch complex, complex member encoding nucleic acids, complex member proteins, and derivatives of the foregoing, and thus, have potential use as agonists or antagonists of Delta:Kuz or D1^{EC}:Notch complex activity or formation. The present invention is thus directed to assays for detecting molecules that specifically bind to, or modulate the function of, Delta, Notch and Kuz nucleic acids, proteins or derivatives of the nucleic acids and proteins. For example, recombinant cells expressing both Delta and Kuz nucleic acids can be used to recombinantly produce the complexes or proteins in these assays, to screen for molecules that bind to, or interfere with, or promote Delta:Kuz complex formation or activity. In preferred embodiments, polypeptide analogs that have superior stabilities but retain the ability to form a Delta:Kuz or D1^{EC}:Notch complex (e.g., Delta and Kuz or D1^{EC} and Notch modified to be resistant to proteolytic degradation in the binding assay buffers, or to be resistant to oxidative

degradation), are used to screen for modulators of Delta activity or Kuz activity or Delta:Kuz complex activity or formation, or are used to screen for modulators of D1^{EC} activity or Notch activity or D1^{EC}:Notch complex activity or formation. Such resistant molecules can be generated, e.g., by substitution of amino acids at proteolytic cleavage sites, the use of chemically derivatized amino acids at proteolytic susceptible sites, and the replacement of amino acid residues subject to oxidation, i.e. methionine and cysteine.

10 A molecule (e.g., a putative binding partner or modulator of Delta:Kuz or D1^{EC}:Notch complex activity or formation) is contacted with the Delta:Kuz or D1^{EC}:Notch complex, or fragment thereof, respectively, under conditions conducive to binding or modulation, and then a molecule that specifically bind to or modulate Delta:Kuz or D1^{EC}:Notch
15 complex activity or formation is identified. Similar methods can be used to screen for molecules that bind to or modulate the function of Delta:Kuz or D1^{EC}:Notch complex encoding nucleic acids or derivatives thereof.

A particular aspect of the present invention
20 relates to identifying molecules that inhibit or promote formation or degradation of a Delta:Kuz or D1^{EC}:Notch complex, e.g., using the method described for screening inhibitors using the modified yeast matrix mating test described in International Patent Publication WO 97/47763 entitled
25 "Identification and Comparison of Protein-Protein Interactions that Occur in Populations and Identification of Inhibitors of These Interactions", which is incorporated by reference herein in its entirety.

In one embodiment of the invention, a molecule that modulates activity of Delta or Kuz, or a complex of Delta and
30 Kuz, is identified by contacting one or more candidate molecules with Delta in the presence of Kuz; and measuring the amount of complex that forms between Delta and Kuz;

wherein an increase or decrease in the amount of complex that forms relative to the amount that forms in the absence of the candidate molecule(s) indicates that the molecule(s) modulates the activity of Delta or Kuz or said complex of Delta and Kuz. In preferred embodiments, a modulator is identified by administering a candidate molecule to a transgenic non-human animal expressing both Delta and Kuz from promoters that are not the native Delta or the native Kuz promoters, more preferably where the candidate molecule is also recombinantly expressed in the transgenic non-human animal. Alternatively, the method for identifying such a modulator can be carried out *in vitro*, preferably with purified Delta, purified Kuz, and a purified candidate molecule.

In another embodiment of the invention, a molecule that modulates activity of Dl^{EC} or Notch, or a complex of Dl^{EC} and Notch, is identified by contacting one or more candidate molecules with Dl^{EC} in the presence of Notch; and measuring the amount of complex that forms between Dl^{EC} and Notch; wherein an increase or decrease in the amount of complex that forms relative to the amount that forms in the absence of the candidate molecule(s) indicates that the molecule(s) modulates the activity of Dl^{EC} or Notch or said complex of Dl^{EC} and Notch. In preferred embodiments, a modulator is identified by administering a candidate molecule to a transgenic non-human animal expressing both Dl^{EC} and Notch from promoters that are not the native Dl^{EC} or the native Notch promoters, more preferably where the candidate molecule is also recombinantly expressed in the transgenic non-human animal. Alternatively, the method for identifying such a modulator can be carried out *in vitro*, preferably with purified Dl^{EC} , purified Notch, and a purified candidate molecule.

Methods that can be used to carry out the foregoing are commonly known in the art. Agents/molecules to be screened can be provided as mixtures of a limited number of specified compounds, or as compound libraries, peptide
5 libraries and the like as described in Section 5.7, *infra*. Agents/molecules to be screened may also include all forms of antisera, antisense nucleic acids, etc., that can modulate complex activity or formation.

5.7 CANDIDATE MOLECULES

10 Any molecule known in the art can be tested for its ability to modulate Delta activation or Kuz function as measured by the expression of one or more of the Delta cleavage products disclosed herein. Furthermore, any molecule known in the art can be tested for its ability to
15 modulate Delta:Kuz complex function, or for its ability to modulate D1^{EC}:Notch complex function. For identifying a molecule that modulates Delta activation or Kuz function, candidate molecules can be directly provided to a cell expressing Delta or Kuz or, in the case of candidate
20 proteins, can be provided by providing their encoding nucleic acids under conditions in which the nucleic acids are recombinantly expressed to produce the candidate proteins within the Delta or Kuz expressing cell. In an embodiment of the invention directed to the assay using full-length Delta
25 and a composition comprising cellular proteins, candidate molecules can also be added to a composition comprising cellular proteins (whole cell lysates, membrane fraction, etc.), preferably derived from cells endogenously or recombinantly expressing Delta.

This embodiment of the invention is well suited to
30 screen chemical libraries for molecules which modulate, e.g., inhibit, antagonize, or agonize, Delta activation or Kuz function or complex function. The chemical libraries can be

peptide libraries, peptidomimetic libraries, other non-peptide synthetic organic libraries, etc.

Exemplary libraries are commercially available from several sources (ArQule, Tripos/PanLabs, ChemDesign, 5 Pharmacopoeia). In some cases, these chemical libraries are generated using combinatorial strategies that encode the identity of each member of the library on a substrate to which the member compound is attached, thus allowing direct and immediate identification of a molecule that is an effective modulator. Thus, in many combinatorial approaches, 10 the position on a plate of a compound specifies that compound's composition. Also, in one example, a single plate position may have from 1-20 chemicals that can be screened by administration to a well containing the interactions of interest. Thus, if modulation is detected, smaller and 15 smaller pools of interacting pairs can be assayed for the modulation activity. By such methods, many candidate molecules can be screened.

Many diversity libraries suitable for use are known in the art and can be used to provide compounds to be tested 20 according to the present invention. Alternatively, libraries can be constructed using standard methods. Chemical (synthetic) libraries, recombinant expression libraries, or polysome-based libraries are exemplary types of libraries that can be used.

The libraries can be constrained or semirigid 25 (having some degree of structural rigidity), or linear or nonconstrained. The library can be a cDNA or genomic expression library, random peptide expression library or a chemically synthesized random peptide library, or non-peptide library. Expression libraries are introduced into the cells 30 in which the assay occurs, where the nucleic acids of the library are expressed to produce their encoded proteins.

In one embodiment, peptide libraries that can be used in the present invention may be libraries that are chemically synthesized *in vitro*. Examples of such libraries are given in Houghten et al., 1991, Nature 354:84-86, which describes mixtures of free hexapeptides in which the first and second residues in each peptide were individually and specifically defined; Lam et al., 1991, Nature 354:82-84, which describes a "one bead, one peptide" approach in which a solid phase split synthesis scheme produced a library of peptides in which each bead in the collection had immobilized thereon a single, random sequence of amino acid residues; Medynski, 1994, Bio/Technology 12:709-710, which describes split synthesis and T-bag synthesis methods; and Gallop et al., 1994, J. Medicinal Chemistry 37(9):1233-1251. Simply by way of other examples, a combinatorial library may be prepared for use, according to the methods of Ohlmeyer et al., 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422-11426; Houghten et al., 1992, Biotechniques 13:412; Jayawickreme et al., 1994, Proc. Natl. Acad. Sci. USA 91:1614-1618; or Salmon et al., 1993, Proc. Natl. Acad. Sci. USA 90:11708-11712. PCT Publication No. WO 93/20242 and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381-5383 describe "encoded combinatorial chemical libraries," that contain oligonucleotide identifiers for each chemical polymer library member.

Further, more general, structurally constrained, organic diversity (e.g., nonpeptide) libraries, can also be used. By way of example, a benzodiazepine library (see e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) may be used.

Conformationally constrained libraries that can be used include but are not limited to those containing invariant cysteine residues which, in an oxidizing

environment, cross-link by disulfide bonds to form cystines, modified peptides (e.g., incorporating fluorine, metals, isotopic labels, are phosphorylated, etc.), peptides containing one or more non-naturally occurring amino acids, non-peptide structures, and peptides containing a significant
5 fraction of γ -carboxyglutamic acid.

Libraries of non-peptides, e.g., peptide derivatives (for example, that contain one or more non-naturally occurring amino acids) can also be used. One
10 example of these are peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371). Peptoids are polymers of non-natural amino acids that have naturally occurring side chains attached not to the alpha carbon but to the backbone amino nitrogen. Since peptoids are not easily degraded by human digestive enzymes, they are advantageously
15 more easily adaptable to drug use. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al., 1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

20 The members of the peptide libraries that can be screened according to the invention are not limited to containing the 20 naturally occurring amino acids. In particular, chemically synthesized libraries and polysome based libraries allow the use of amino acids in addition to the 20 naturally occurring amino acids (by their inclusion in
25 the precursor pool of amino acids used in library production). In specific embodiments, the library members contain one or more non-natural or non-classical amino acids or cyclic peptides. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids,
30 α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid; γ -Abu, e-Ahx, 6-amino hexanoic acid; Aib, 2-amino isobutyric acid; 3-amino propionic acid; ornithine;

norleucine; norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, designer amino acids such as β -methyl amino acids, $\text{C}\alpha$ -methyl amino acids, $\text{N}\alpha$ -methyl amino acids, fluoro-amino acids and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Further, toporythmic proteins, derivatives and fragments thereof, can be tested for the ability to modulate Delta activation. Toporythmic proteins, and more generally, members of the "Notch cascade" or the "Notch group" of genes, include Notch, Delta, Serrate, Kuz, and other members of the Delta/Serrate family, which are identified by genetic (as detected phenotypically, e.g., in *Drosophila*) or molecular interaction (e.g., binding *in vitro*). See, International Publications WO 92/19734, WO 97/18822, WO 96/27610, and WO 97/01571 and references therein, for examples of vertebrate and non-vertebrate members of the Notch family of genes.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390; Fowlkes et al., 1992, BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al., 1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-566; Tuerk et al., 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Patent No. 5,096,815, U.S. Patent No. 5,223,409, and U.S. Patent No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, Science 263:671-673; and International Patent Publication No. WO 94/18318.

In a specific embodiment, screening can be carried out by contacting the library members with Delta or Kuz or a

protein complex or the present invention (or encoding nucleic acid or derivative) immobilized on a solid phase, and harvesting those library members that bind to the protein or complex (or encoding nucleic acid or derivative). Examples
5 of such screening methods, termed "panning" techniques, are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; International Patent Publication No. WO 94/18318; and in references cited hereinabove.

10 In a specific embodiment, fragments and/or analogs of Delta or Kuz, especially peptidomimetics, are screened for activity as competitive or non-competitive inhibitors of Delta:Kuz complex formation, which thereby inhibit Delta:Kuz complex activity or formation.

Methods for screening may involve labeling the
15 proteins or complex proteins of the present invention with radioligands (e.g., ^{125}I or ^3H), magnetic ligands (e.g., paramagnetic beads covalently attached to photobiotin acetate), fluorescent ligands (e.g., fluorescein or rhodamine), or enzyme ligands (e.g., luciferase or beta-
20 galactosidase). The reactants that bind in solution can then be isolated by one of many techniques known in the art, including but not restricted to, co-immunoprecipitation of the labeled protein or complex moiety using antisera against the unlabeled binding partner (or labeled binding partner
25 labeled protein or complex moiety), immunoaffinity chromatography, size exclusion chromatography, and gradient density centrifugation. In a preferred embodiment, the labeled binding partner is a small fragment or peptidomimetic that is not retained by a commercially available filter.
30 Upon binding, the labeled species is then unable to pass through the filter, providing for a simple assay of complex formation.

Methods commonly known in the art are used to label proteins. Suitable labeling methods include, but are not limited to, radiolabeling by incorporation of radiolabeled amino acids, e.g., ^3H -leucine or ^{35}S -methionine, radiolabeling
5 by post-translational iodination with ^{125}I or ^{131}I using the chloramine T method, Bolton-Hunter reagents, etc., or labeling with ^{32}P using phosphorylase and inorganic radiolabeled phosphorous, biotin labeling with photobiotin-acetate and sunlamp exposure, etc.

10

5.8 THERAPEUTIC USES

The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta
15 cleavage peptides, Delta:Kuz and Dl^{EC} :Notch protein complexes and analogs and derivatives (including fragments) thereof (e.g., as described hereinabove); antibodies thereto (as described hereinabove); nucleic acids encoding the Delta cleavage peptides, analogs, or derivatives (e.g., as
20 described hereinabove) as well as the protein complexes of the present invention; and *Delta*, *Notch* and *Kuz* antisense nucleic acids. In addition, such Therapeutics include soluble Delta peptides and derivatives and analogs thereof, antibodies thereto, nucleic acids encoding the soluble Delta
25 peptides, derivatives, or analogs, and soluble Delta peptide antisense nucleic acids. In a particular embodiment, the Therapeutic is a peptide comprising a fragment of a Delta protein of about amino acid Cys_{516} to about amino acid Phe_{543} in human Delta (SEQ ID NO:10), of about amino acid Cys_{515} to about amino acid Phe_{543} in mouse Delta (SEQ ID NO:6), of about
30 amino acid Cys_{523} to about amino acid Phe_{551} in chick Delta (SEQ ID NO:7), of about amino acid Cys_{518} to about amino acid Phe_{544} in *Xenopus* Delta (SEQ ID NO:8), and the sequence of

about amino acid Cys₅₆₄ to about amino acid Ala₅₉₃ or Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9). In specific embodiments, the peptide comprises 25, 30, 35, 40, 50, 100, 150, 200 or 250 contiguous amino acids of a Delta protein. Antagonist

5 Therapeutics of the invention are those Therapeutics which antagonize, or inhibit, Delta function and/or Notch function (since Delta is a Notch ligand) and/or Kuz function (since Kuz binds to and proteolytically processes Delta). Such Antagonist Therapeutics are most preferably identified by use of known convenient *in vitro* assays, e.g., based on their

10 ability to inhibit binding of Delta to another protein (e.g., a Notch protein or a Kuz protein), or inhibit any known Notch or Delta or Kuz function as preferably assayed *in vitro* or in cell culture, although genetic assays (e.g., in *Drosophila*) may also be employed. In a preferred embodiment, the

15 Antagonist Therapeutic is a Delta cleavage peptide which mediates binding to Kuz, or an antibody thereto. In other specific embodiments, such an Antagonist Therapeutic is a nucleic acid capable of expressing a molecule comprising a Delta cleavage peptide which binds to Kuz, or a Delta

20 antisense nucleic acid (see Section 5.11 herein). It should be noted that preferably, suitable *in vitro* or *in vivo* assays, as described *infra*, should be utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected

25 tissue, since the developmental history of the tissue may determine whether an Antagonist or Agonist Therapeutic is desired.

In addition, the mode of administration, e.g., whether administered in soluble form or administered via its encoding nucleic acid for intracellular recombinant

30 expression, of the Delta cleavage peptide or derivative or protein complex or derivative can affect whether it acts as an agonist or antagonist.

The Agonist Therapeutics of the invention, as described *supra*, promote Delta function or Notch function or Kuz function. Such Agonist Therapeutics include but are not limited to proteins and derivatives comprising the portions
5 of Delta that mediate binding to Kuz, and nucleic acids encoding the foregoing (which can be administered to express their encoded products *in vivo*).

Further descriptions and sources of Therapeutics of the inventions are found in Sections 5.1 through 5.7 herein.

10 Molecules which retain, or alternatively inhibit, a desired Delta property, e.g., binding to Kuz, binding to an intracellular ligand, can be used therapeutically as inducers, or inhibitors, respectively, of such property and its physiological correlates. In a specific embodiment, a peptide (e.g., in the range of 6-50 or 100-200 amino acids;
15 and particularly of about 25, 30, 35, 50, 100 or 150 amino acids) containing the sequence of a portion of Delta which binds to Kuz is used to antagonize Delta or Notch function. In a specific embodiment, such an Antagonist Therapeutic is used to treat or prevent human or other malignancies
20 associated with increased Notch expression (e.g., cervical cancer, colon cancer, breast cancer, squamous adenocarcinomas (see *infra*)). Derivatives or analogs of Delta can be tested for the desired activity by procedures known in the art, including but not limited to the assays described in the
25 examples *infra*. In one specific embodiment, peptide libraries can be screened to select a peptide with the desired activity; such screening can be carried out by assaying, e.g., for binding to Kuz.

Other Therapeutics include molecules that bind to a Kuz. Thus, the invention also provides a method for
30 identifying such molecules. Such molecules can be identified by a method comprising contacting a plurality of molecules (e.g., in a peptide library, or combinatorial chemical

library) with the Kuz protein under conditions conducive to binding, and recovering any molecules that bind to the Kuz protein.

The Agonist and Antagonist Therapeutics of the invention have therapeutic utility for disorders of cell fate. The Agonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an absence or decreased (relative to normal, or desired) levels of Notch or Delta or Kuz function, for example, in patients where Delta protein is lacking, genetically defective, biologically inactive or underactive, or underexpressed; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays (see *infra*) indicate the utility of Delta agonist administration. The absence or decreased levels in Notch or Delta or Kuz function can be readily detected, e.g., by obtaining a patient tissue sample (e.g., from biopsy tissue) and assaying it *in vitro* for protein levels, structure and/or activity of the expressed Notch or Delta or Kuz protein. Many methods standard in the art can be thus employed, including but not limited to immunoassays to detect and/or visualize Notch or Delta or Kuz protein (e.g., Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect Notch or Delta or Kuz expression by detecting and/or visualizing respectively Notch or Delta or Kuz mRNA (e.g., Northern assays, dot blots, *in situ* hybridization, etc.)

In vitro assays which can be used to determine whether administration of a specific Agonist Therapeutic or Antagonist Therapeutic is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the

patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant cells (e.g., by promoting terminal differentiation) is selected for therapeutic use *in vivo*. Many assays standard in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ³H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (e.g., *fos*, *myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc. In a specific aspect, the malignant cell cultures are separately exposed to (1) an Agonist Therapeutic, and (2) an Antagonist Therapeutic; the result of the assay can indicate which type of Therapeutic has therapeutic efficacy.

In another embodiment, a Therapeutic is indicated for use which exhibits the desired effect, inhibition or promotion of cell growth, upon a patient cell sample from tissue having or suspected of having a hyper- or hypoproliferative disorder, respectively. Such hyper- or hypoproliferative disorders include but are not limited to those described in Sections 5.8.1 through 5.8.3 *infra*.

In another specific embodiment, a Therapeutic is indicated for use in treating nerve injury or a nervous system degenerative disorder (see Section 5.8.2) which exhibits *in vitro* promotion of nerve regeneration/neurite extension from nerve cells of the affected patient type.

In addition, administration of an Antagonist Therapeutic of the invention is also indicated in diseases or disorders determined or known to involve a Notch or Delta or Kuz dominant activated phenotype ("gain of function")

mutations.) Administration of an Agonist Therapeutic is indicated in diseases or disorders determined or known to involve a Notch or Delta or Kuz dominant negative phenotype ("loss of function" mutations). The functions of various structural domains of the Notch protein have been investigated *in vivo*, by ectopically expressing a series of *Drosophila Notch* deletion mutants under the hsp70 heat-shock promoter, as well as eye-specific promoters (see Rebay et al., 1993, Cell 74:319-329). Two classes of dominant phenotypes were observed, one suggestive of Notch loss-of function mutations and the other of Notch gain-of-function mutations. Dominant "activated" phenotypes resulted from overexpression of a protein lacking most extracellular sequences, while dominant "negative" phenotypes resulted from overexpression of a protein lacking most intracellular sequences. The results indicated that Notch functions as a receptor whose extracellular domain mediates ligand-binding, resulting in the transmission of developmental signals by the cytoplasmic domain.

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (*i.e.*, less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed phenotype) is selected for therapeutic use. Many assays standard in the art can be used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present. For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics

associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar
5 transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather
10 than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic or pre-neoplastic disorder desired to be treated or prevented, or is derived from the neural or other cell type
15 upon which an effect is desired, according to the present invention.

The Antagonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving increased (relative to normal, or
20 desired) levels of Notch or Delta or Kuz function, for example, where the Notch or Delta or Kuz protein is overexpressed or overactive; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays indicate the utility of Delta antagonist administration. The increased levels of
25 Notch or Delta or Kuz function can be readily detected by methods such as those described above, by quantifying protein and/or RNA. *In vitro* assays with cells of patient tissue sample or the appropriate cell line or cell type, to determine therapeutic utility, can be carried out as
described above.

30

5.8.1 MALIGNANCIES

Malignant and pre-neoplastic conditions which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be
 5 treated upon thus observing an indication of therapeutic utility, include but are not limited to those described below in Sections 5.8.1 and 5.9.1.

Malignancies and related disorders, cells of which type can be tested *in vitro* (and/or *in vivo*), and upon
 10 observing the appropriate assay result, treated according to the present invention, include but are not limited to those listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

15

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

	Leukemia
	acute leukemia
20	acute lymphocytic leukemia
	acute myelocytic leukemia
	myeloblastic
	promyelocytic
	myelomonocytic
	monocytic
	erythroleukemia
	chronic leukemia
25	chronic myelocytic (granulocytic) leukemia
	chronic lymphocytic leukemia
	Polycythemia vera
	Lymphoma
	Hodgkin's disease
	non-Hodgkin's disease
	Multiple myeloma
	Waldenström's macroglobulinemia
30	Heavy chain disease
	Solid tumors
	sarcomas and carcinomas
	fibrosarcoma
	myxosarcoma

liposarcoma
chondrosarcoma
osteogenic sarcoma
chordoma
angiosarcoma
endotheliosarcoma
5 lymphangiosarcoma
lymphangioendotheliosarcoma
synovioma
mesothelioma
Ewing's tumor
leiomyosarcoma
rhabdomyosarcoma
colon carcinoma
10 pancreatic cancer
breast cancer
ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
15 sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
20 bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
testicular tumor
lung carcinoma
25 small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
30 pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
menangioma

melanoma
neuroblastoma
retinoblastoma

5 In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias) are treated or prevented in epithelial tissues such as those in the cervix, esophagus, and lung.

 Malignancies of the colon and cervix exhibit
10 increased expression of human Notch relative to such non-malignant tissue (see PCT Publication no. WO 94/07474 published April 14, 1994, incorporated by reference herein in its entirety). Thus, in specific embodiments, malignancies or premalignant changes of the colon or cervix are treated or prevented by administering an effective amount of an
15 Antagonist Therapeutic, e.g., a Delta cleavage peptide, that antagonizes Notch function. The presence of increased Notch expression in colon, and cervical cancer suggests that many more cancerous and hyperproliferative conditions exhibit upregulated Notch. Thus, in specific embodiments, various
20 cancers, e.g., breast cancer, squamous adenocarcinoma, seminoma, melanoma, and lung cancer, and premalignant changes therein, as well as other hyperproliferative disorders, can be treated or prevented by administration of an Antagonist Therapeutic that antagonizes Notch function.

25

5.8.2 NERVOUS SYSTEM DISORDERS

 Nervous system disorders, involving cell types which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and
30 which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration

of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the
5 central (including spinal cord, brain) or peripheral nervous systems:

- (i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
- 10 (ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
- 15 (iii) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue;
- 20 (iv) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease,
- 25 tuberculosis, syphilis;
- (v) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated
30 with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

- 5 (vi) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;
- 10 (vii) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;
- 15 (viii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and
- (ix) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not
- 20 limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

25 Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons (see also Section 5.8). For example, and not by way of limitation, Therapeutics which elicit any of the following effects may be

30 useful according to the invention:

- (i) increased survival time of neurons in culture;

- (ii) increased sprouting of neurons in culture or *in vivo*;
- (iii) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or
- (iv) decreased symptoms of neuron dysfunction in *vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In a specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio

syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.8.3 TISSUE REPAIR AND REGENERATION

5 In another embodiment of the invention, a Therapeutic of the invention is used for promotion of tissue regeneration and repair, including but not limited to treatment of benign dysproliferative disorders. Specific
10 embodiments are directed to treatment of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid
(hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate
15 determination), and baldness (a condition in which terminally differentiated hair follicles (a tissue rich in Notch) fail to function properly). In another embodiment, a Therapeutic of the invention is used to treat degenerative or traumatic disorders of the sensory epithelium of the inner ear.

20

5.9 PROPHYLACTIC USES

5.9.1 MALIGNANCIES

The Therapeutics of the invention can be administered to prevent progression to a neoplastic or
25 malignant state, including but not limited to those disorders listed in Table 1. Such administration is indicated where the Therapeutic is shown in assays, as described *supra*, to have utility for treatment or prevention of such disorder. Such prophylactic use is indicated in conditions known or
30 suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see

Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. As mentioned *supra*, such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface

protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the
5 epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign
10 epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a
Therapeutic: a chromosomal translocation associated with a
15 malignancy (e.g., the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree
20 kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (e.g., familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and
25 pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; see Robbins and Angell, 1976, *Basic Pathology*, 2d
30 Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, an Antagonist Therapeutic of the invention is administered to a human

patient to prevent progression to breast, colon, or cervical cancer.

5.9.2 OTHER DISORDERS

5 In other embodiments, a Therapeutic of the invention can be administered to prevent a nervous system disorder described in Section 5.8.2, or other disorder (e.g., liver cirrhosis, psoriasis, keloids, baldness) described in Section 5.8.3.

10

5.10 DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

The Therapeutics of the invention can be tested *in vivo* for the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal
15 model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

20

5.11 USE OF ANTISENSE OLIGONUCLEOTIDES FOR SUPPRESSION OF DELTA ACTIVATION OR DELTA:KUZ OR D1^{EC}:NOTCH COMPLEX ACTIVITY OR FORMATION

In a specific embodiment of the present invention, Delta cleavage peptide, Delta, Kuz, Notch, and Delta:Kuz or D1^{EC}:Notch complex activity and/or formation, is inhibited by
25 use of antisense nucleic acids for Delta, Notch and/or Kuz. The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding Delta, Notch and/or Kuz, or a portion thereof. An "antisense" nucleic
30 acid as used herein refers to a nucleic acid capable of hybridizing to a portion of a Delta, Notch or Kuz RNA (preferably mRNA) by virtue of some sequence complementarily. The antisense nucleic acid may be complementary to a coding

and/or noncoding region of a Delta, Notch or Kuz mRNA. Such antisense nucleic acids that inhibit Delta cleavage peptide activity or Delta:Kuz complex formation or activity or D1^{EC}:Notch complex formation or activity have utility as
5 Therapeutics, and can be used in the treatment or prevention of disorders as described, *supra*.

The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA, or a modification or derivative thereof, which
10 can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

In another embodiment, the present invention is directed to a method for inhibiting the expression of Delta cleavage peptide nucleic acid sequences, in a prokaryotic or
15 eukaryotic cell, comprising providing the cell with an effective amount of a composition comprising an antisense nucleic acid of Delta cleavage peptide, or a derivative thereof, of the invention.

The antisense nucleic acids are of at least six
20 nucleotides and are preferably oligonucleotides, ranging from 6 to about 200 nucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or
25 chimeric mixtures, or derivatives or modified versions thereof, and either single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents
30 Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; International Patent Publication No. WO 88/09810)

or blood-brain barrier (see, e.g., International Patent Publication No. WO 89/10134), hybridization-triggered cleavage agents (see, e.g., Krol et al., 1988, BioTechniques 6:958-976), or intercalating agents (see, e.g., Zon, 1988, 5 Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a Delta cleavage peptide antisense oligonucleotide is provided, preferably as single-stranded DNA. The oligonucleotide may be modified at any position in its structure with constituents generally known in the art.

10 The antisense oligonucleotides may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 15 5-carboxymethylaminomethyl-2-thio-uridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 20 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5N-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methyl-thio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, 25 queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide 30 comprises at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal, or an analog of the foregoing.

In yet another embodiment, the oligonucleotide is a 2- α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization-triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligo-nucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

In a specific embodiment, the antisense oligonucleotides comprise catalytic RNAs, or ribozymes (see, e.g., International Patent Publication No. WO 90/11364; Sarver et al., 1990, Science 247:1222-1225). In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res.

15:6131-6148), or a chimeric RNA-DNA analog (Inoue et al., 1987, FEBS Lett. 215:327-330).

In an alternative embodiment, the antisense nucleic acids of the invention are produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced in vivo such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art to be capable of replication and expression in mammalian cells. Expression of the sequences encoding the antisense RNAs can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a Delta, Notch or Kuz gene, preferably a human Delta, Notch or Kuz gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming

a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

The antisense nucleic acid can be used to treat (or prevent) disorders of a cell type that expresses, or preferably overexpresses, the Delta cleavage peptide or the Delta:Kuz complex or the DL^{EC} :Notch complex. In a preferred embodiment, a single-stranded Delta, Notch or Kuz DNA antisense oligonucleotide, both single-stranded Delta, Notch and Kuz antisense oligonucleotides, or a single-stranded Delta:Kuz DNA antisense fusion sequence, is used.

Cell types that express or overexpress Delta, Notch and/or Kuz RNA can be identified by various methods known in the art. Such methods include, but are not limited to, hybridization with Delta-, Notch- and Kuz-specific nucleic acids (e.g., by Northern blot hybridization, dot blot hybridization, or in situ hybridization), or by observing the ability of RNA from the cell type to be translated in vitro into Delta or Kuz by immunohistochemistry, Western blot analysis, ELISA, etc. In a preferred aspect, primary tissue from a patient can be assayed for Delta, Notch and/or Kuz expression prior to treatment, e.g., by immunocytochemistry, in situ hybridization, or any number of methods to detect protein or mRNA expression.

Pharmaceutical compositions of the invention (see Section 5.7, *infra*), comprising an effective amount of an

antisense nucleic acid in a pharmaceutically acceptable carrier can be administered to a patient having a disease or disorder that is of a type that expresses or overexpresses, for example a Delta:Kuz complex.

5 The amount of an antisense nucleic acid that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity in vitro, and then in useful animal
10 model systems, prior to testing and use in humans.

 In a specific embodiment, pharmaceutical compositions comprising Delta and Kuz antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it
15 may be useful to use such compositions to achieve sustained release of the Delta and/or Kuz antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable central nervous system cell types (Leonetti et al., 1990,
20 Proc. Natl. Acad. Sci. U.S.A. 87:2448-2451; Renneisen et al., 1990, J. Biol. Chem. 265:16337-16342).

5.12 THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

 The invention provides methods of treatment (and
25 prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably
30 a mammal, and most preferably human.

 Various delivery systems are known and can be used to administer a Therapeutic of the invention, e.g.,

encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as
5 part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or
10 mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention
15 into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary
20 administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be
25 achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous,
30 non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or

former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that

it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface
 5 receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid
 Therapeutic can be introduced intracellularly and
 10 incorporated within host cell DNA for expression, by homologous recombination.

In specific embodiments directed to treatment or prevention of particular disorders, preferably the following forms of administration are used:

15 <u>Disorder</u>	<u>Preferred Forms of Administration</u>
Cervical cancer	Topical
Gastrointestinal cancer	Oral; intravenous
Lung cancer	Inhaled; intravenous
Leukemia	Intravenous; extracorporeal
Metastatic carcinomas	Intravenous; oral
20 Brain cancer	Targeted; intravenous; intrathecal
Liver cirrhosis	Oral; intravenous
Psoriasis	Topical
Keloids	Topical
Baldness	Topical
25 Spinal cord injury	Targeted; intravenous; intrathecal
Parkinson's disease	Targeted; intravenous; intrathecal
Motor neuron disease	Targeted; intravenous; intrathecal
Alzheimer's disease	Targeted; intravenous; intrathecal

30 The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically

acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to

provide the form for proper administration to the patient.
The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard

clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the
5 seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. .
10 Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

Suppositories generally contain active ingredient
15 in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of
20 the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human
25 administration.

5.13 DIAGNOSTIC UTILITY

Delta cleavage peptides, soluble Delta peptides, analogs, derivatives, and subsequences thereof, Delta
cleavage peptide encoding nucleic acids (and sequences
30 complementary thereto), soluble Delta peptide encoding nucleic acids (and sequences complementary thereto), anti-Delta cleavage peptide antibodies, anti-soluble Delta peptide

antibodies, and anti-Delta:Kuz and anti-Dl^{EC}:Notch complex. antibodies have uses in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting Delta cleavage peptide expression, or monitor the treatment thereof. In a particular example, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti-Delta cleavage peptide antibody under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, preferably in conjunction with binding of anti-Kuz or anti-Notch antibody can be used to detect aberrant Delta, Notch and/or Kuz localization or aberrant levels of Dl^{EC}:Notch or Delta-Kuz colocalization in a disease state. In a specific embodiment, antibody to Delta cleavage peptide can be used to assay in a patient tissue or serum sample for the presence of Delta cleavage peptide where an aberrant level of Delta cleavage peptide is an indication of a diseased condition. Aberrant levels of Delta binding ability in an endogenous Notch or Kuz protein, or aberrant levels of binding ability to Kuz (or other Delta ligand, e.g., Notch) in an endogenous Delta cleavage peptide may be indicative of a disorder of cell fate (e.g., cancer, etc.) By "aberrant levels," is meant increased or decreased levels relative to that present, or a standard level representing that present, in an analogous sample from a portion of the body or from a subject not having the disorder.

The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin

reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

5 Delta, Notch and Kuz genes and related nucleic acid sequences and subsequences, including complementary sequences, and other toporythmic gene sequences, can also be used in hybridization assays. Delta, Notch and Kuz nucleic acid sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes.

10 Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in Delta expression and/or activity as described *supra*. In particular, such a hybridization assay is carried out by a method comprising

15 contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to Delta, Notch or Kuz DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

 Additionally, since Delta binds to Notch and Kuz,

20 Delta or a binding portion thereof can be used to assay for the presence and/or amounts of Notch or Kuz in a sample, e.g., in screening for malignancies which exhibit increased Notch expression such as colon and cervical cancers.

25 5.14 ANIMAL MODELS

 The present invention also provides animal models. In one embodiment, animal models for diseases and disorders involving Delta cleavage peptide, soluble Delta peptide, and Delta:Kuz and D1^{EC}:Notch complexes are provided. These include, but are not limited to, disorders of cell fate and

30 differentiation such as cancer. Such animals can be initially produced by promoting homologous recombination or insertional mutagenesis between Delta, Notch and Kuz genes in

the chromosome, and exogenous *Delta*, *Notch* and *Kuz* genes that have been rendered biologically inactive or deleted (preferably by insertion of a heterologous sequence, e.g., an antibiotic resistance gene). In a preferred aspect, 5 homologous recombination is carried out by transforming embryo-derived stem (ES) cells with a vector containing, e.g., the insertionally inactivated *Delta* and *Kuz* gene, such that homologous recombination occurs, followed by injecting the transformed ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of 10 the chimeric animal ("knockout animal") in which a *Delta* and/or *Kuz* gene has been inactivated or deleted (Capecchi, 1989, *Science* 244:1288-1292). The chimeric animal can be bred to produce additional knockout animals. Such animals can be mice, hamsters, sheep, pigs, cattle, etc., and are 15 preferably non-human mammals. In a specific embodiment, a knockout mouse is produced.

Such knockout animals are expected to develop, or be predisposed to developing, diseases or disorders involving, but not restricted to, disorders of cell fate and 20 differentiation, etc., and thus, can have use as animal models of such diseases and disorders, e.g., to screen for or test molecules (e.g., potential Therapeutics) for disorders of cell fate and differentiation, and other diseases.

In a different embodiment of the invention, 25 transgenic animals that have incorporated and express (or overexpress or mis-express) a functional *Delta* and/or *Kuz* gene, e.g. by introducing the *Delta* and *Kuz* genes under the control of a heterologous promoter (i.e., a promoter that is not the native *Delta* or *Kuz* promoter) that either overexpresses the protein or proteins, or expresses them in 30 tissues not normally expressing the complexes or proteins, can have use as animal models of diseases and disorders characterized by elevated levels of *Delta*:*Kuz* complexes.

Such animals can be used to screen or test molecules for the ability to treat or prevent the diseases and disorders cited *supra*.

In one embodiment, the present invention provides a recombinant non-human animal in which both an endogenous Delta gene and an endogenous Kuz have been deleted or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof. In another embodiment, the invention provides a recombinant non-human animal containing both a Delta gene and a Kuz gene in which the Delta gene is under the control of a promoter that is not the native Kuz gene promoter and the Kuz gene is under the control of a promoter that is not the native Kuz gene promoter. In a specific embodiment, the invention provides a recombinant non-human animal containing a transgene comprising a nucleic acid sequence encoding a chimeric protein comprising a Delta cleavage peptide of at least 6 amino acids fused via a covalent bond to a fragment of Kuz protein of at least 6 amino acids.

20 6. THE NOTCH LIGAND DELTA IS CLEAVED BY THE DISINTEGRIN METALLOPROTEASE KUZBANIAN

The Notch signaling pathway defines an evolutionary conserved cell interaction mechanism which throughout development controls the fate of cells by modulating their response to developmental signals (Artavanis-Tsakonas et al., 1995, Science 268:225-232; Fleming et al., 1998, Trends in Cell Biology 7:437-441). The Notch receptor is cleaved in the trans-Golgi network as it traffics towards the plasma membrane eventually forming a ligand competent, heterodimeric molecule (Blaumueller et al., 1997, Cell 90:281-291). Both known ligands, Delta and Serrate are thought to act as transmembrane proteins interacting via their extracellular domains with the receptor expressed on adjacent cells

(Fleming et al., 1998, Trends in Cell Biology 7:437-441; Muskavitch, 1994, Developmental Biology 166:415-430). Given the similar phenotypes between loss of Notch signaling and loss of function mutations in the Kuzbanian (Kuz) gene, a
5 gene encoding a putative member of the ADAM family of metalloproteases (Rooke et al., 1996, Nature 273:1227-1231), it has been suggested that Kuz may be involved in the cleavage of the Notch receptor (Pan and Rubin, 1997, Cell 90:271-280. This hypothesis is not corroborated by recent
10 biochemical studies which indicate that the functionally crucial cleavage of Notch in the trans Golgi network is catalyzed by a furin-like convertase (Logeat, et al., 1998, Proc. Nat. Acad. Sci. USA 95:8108-8112). Consistent with this, furin is known to act in this sub-cellular compartment, as opposed to ADAM proteases, such as Kuz, which are thought
15 to act on the cell surface (Wolfsberg et al., 1995, Journal of Cell Biology 131:275-278).

A genetic screen aimed in identifying modifiers of the phenotypes associated with the constitutive expression of a dominant negative transgene of Kuz (KuzDN) in developing
20 imaginal discs, has uncovered Delta as an interacting gene (Wu et al., unpublished observation). Flies expressing this dominant negative construct, even though they also carry a wild type complement of Kuz become semi-lethal when heterozygous for a loss of function Delta mutation (Xu et
25 al., unpublished observation). In contrast, Delta duplications rescue the phenotypes associated with KuzDN (Figures 6A-6F). KuzDN flies display extra vein material, especially deltas, at the ends of the longitudinal veins, wing notching (observed with a low penetrance), extra
30 bristles on the notum, and have small, rough eyes (Figures 6A and 6E). When KuzDN flies carry three, as opposed to the normal two, copies of wild type Notch, the bristle and eye phenotype are not affected (Xu et al., unpublished

observation), nor are the vein deltas altered (Figure 6D).
On the other hand, the KuzDN phenotypes are effectively
suppressed by Delta duplications (Figures 6B and 6F).
Indicating that a higher copy number of Delta molecules is
5 capable of overriding the effect of the KuzDN construct.

The interaction between Delta and Kuz was further
explored by examining the relationship between the protein
products of their respective genes. A monoclonal antibody
was raised against an extracellular Delta epitope generated
by using a fusion protein generated by using a PCR product of
10 the the entire extracellular domain of Drosophila Delta using
the primers 5' GAGTTGCGCCTGAAGTACTT 3' (SEQ ID NO:14) and 5'
GGTCGCTCCATATTGGTGGG 3' (SEQ ID NO:15) and subsequent cloning
into the SmaI site of pGEX3 and StuI site of pMAL. A
monoclonal cell line (C594.9B, designated "9B") was created
15 by standard protocols and screening of hybridoma supernatants
was done by immunostaining of Delta expressing S2 cells.
Ascites fluid was made and used at 1/3000-1/10,000 dilution
for western blotting followed by detection with peroxidase
labelled anti-mouse antibody and chemiluminescent development
20 with a luminol substrate (see Rand et al., 1997, Protein
Science 6:2059-2071). Using this antibody, the Delta
antigen in S2 cells, which stably express full length Delta,
was examined (Figure 7A). S2 cells are known to express wild
type Kuz endogenously (Pan and Rubin, 1997, Cell 90:271-280).
The presence of an immunoreactive fragment in the culture
25 media that migrated faster than full length Delta was
observed exclusively in the media. It is noted that this
fragment, as with full length Delta, was fully 40-fold more
immunoreactive with 9B under non-reducing conditions. Full
length Delta is associated with the cell pellet whereas, this
30 fragment is almost exclusively in the media suggesting it is
a soluble, proteolytic fragment derived from full length
Delta (herein referred to as "Dl^{EC}"). The size of this

fragment under reducing conditions is approximately 67,000 Daltons, consistent the extracellular domain of Delta, which is estimated to be 65,000 Daltons (Figure 7D). D1^{EC} was subsequently affinity purified from the culture medium and subjected to amino acid sequence analysis to determine the N-terminal amino acid sequence. Briefly, *Drosophila* S2 cells expressing Delta were induced with 0.7 mM CuSO₄ in serum free media for two days and the media was collected and precipitated with 70% ammonium sulfate. The precipitate was collected by centrifugation and subsequently resuspended and dialyzed against 20 mM HEPES, 150 mM NaCl, 2 mM CaCl₂, pH 7.4. This sample was passed over Sepharose beads coupled with monoclonal antibody 9B, washed with 1.0 M NaCl and eluted with 25 mM glycine, pH 2.8 and immediately neutralized with 1.0 M Tris-HCl. N-terminal amino acid analysis was performed with an Applied Biosystems gas phase amino acid sequencer.

The amino acid sequence of D1^{EC} is consistent with the predicted polypeptide processing site and is conserved among the *Drosophila*, *Xenopus* and human Delta homologs (Figure 7E).

It is concluded that full length Delta in S2 cells is cleaved at the surface to release a fragment containing most or all of the extracellular domain of Delta (D1^{EC}). Western blot analysis of *Drosophila* embryos reveals the existence of both full length Delta (D1^{FL}) and a fragment with the same mobility as D1^{EC} implicating this same Delta derived product is present *in vivo* (Figure 7B). It is noted that between D1^{FL} and D1^{EC} additional potentially transient proteolytic products are detectable with the 9B antibody (Figure 7B, lane "10 embryos" and Figure 8D, lane "kuz +/-").

The possibility that the generation of D1^{EC} can be influenced by Kuz was examined by cotransfection experiments in S2 cells which, as mentioned earlier, are known to express

wild type Kuz endogenously (Pan and Rubin, 1997, Cell 90:271-280). In transient transfections, cotransfection of Delta with Kuz showed a remarkable increase in the Dl^{EC} fragment in the culture medium compared to Delta transfection alone (Figure 8A). This increase in Dl^{EC} corresponds to a decrease in Dl^{FL} consistent with the notion that Dl^{FL} is the precursor of the Dl^{EC} product. In addition, these data indicate that transfection of Kuz acts additively to the endogenous Kuz in the S2 cells. Supporting this hypothesis, cotransfection with KuzDN has a dramatic inhibitory effect on Dl^{EC} production (Figure 8A). Under identical experimental conditions cotransfection of Kuz or KuzDN has no effect on the proteolytic processing of Notch (Figure 8B). These observations demonstrate that Kuz plays a prominent role in the processing of Delta, one that is not as clear in the processing of Notch. In agreement with this conclusion, it has been found Dl^{EC} production was markedly inhibited by the metalloprotease inhibitors EDTA and 1,10-phenanthroline (Figure 8C), while no effect was observed with serine protease inhibitors (PMSF and aprotinin), cysteine protease inhibitor (leupeptin) or aspartyl protease inhibitor (pepstatin).

With the Dl^{EC} product showing to be present in embryos (Figure 7B), we sought to examine the role of Kuz in generating this product *in vivo*. *kuz* maternal null embryos with either one (*kuz +/-*) or no (*kuz -/-*) zygotic copies of *kuz* were created by crossing female flies carrying *kuz* germline clones with *kuz +/-* male flies (Rooke et al., 1996, Nature 273:1227-1231). *kuz -/-* embryos were clearly distinguished from *kuz +/-* embryos by the absence of malpighian tubules and lack of movement. Extracts prepared from a collection of nine of each type of embryo show the distinct absence of the Dl^{EC} and higher levels of Dl^{FL} in the *Kuz -/-* embryos as compared to *Kuz +/-* (Figure 8D). Re-

probing the same membrane with anti-Notch antibody showed no difference in processing of Notch in the Kuz +/- and Kuz -/- embryos. Furthermore, analysis of 14 randomly selected individual embryos showed eight embryos having significantly
5 higher levels of D1^{FL}, analogous to the kuz -/- embryos (Figure 8D) and consistent with the predicted outcome of the cross. These observations indicate that Kuz mediated the proteolytic processing of Delta *in vivo*.

Although kuz mutations have multiple defects
10 indicating an involvement in different processes (Rooke et al., 1996, Nature 273:1227-1231), its phenotype partially overlaps with that of Delta. Inactivation of kuz during embryogenesis causes a more extensive neurogenic phenotype than Delta mutations, nevertheless, it is clear that in the ventrolateral region the neural hypertrophy in the two
15 mutations is identical. In adult mosaic clones, a small percentage of kuz mutant cells on the clone border develop into multiple bristles (Rooke et al., 1996, Nature 273:1227-1231). Delta mosaic clones present a more complicated situation. While cells on the border of the clones mutant
20 for weak delta alleles commit to epidermal fate, it is evident that cells mutant for strong delta alleles will develop multiple bristles at a low frequency (Figure 3 in Heitzler and Simpson, 1991, Cell, 1083-1092), the phenotype observed in kuz mutants. It is clear, however, that with
25 strong kuz and delta alleles, all extra neurons derive from genotypically mutant cells.

The above observations are distinct from a second function of kuz which has been termed neural promotion function (Rooke et al., 1996, Science 273:1227-1231; Rooke
30 and Xu, 1998, Bioassays 20:209-214). This function prevents cells in the center of kuz clones to develop bristles in contrast to the multiple bristle phenotype of delta clones. The genetic data, including the mosaic analyses, are

compatible with the hypothesis that the processing of Delta protein is mediated by Kuz. These findings are also compatible with earlier genetic studies linking kuz with Notch activity (Pan and Rubin, 1997, Cell 90:271-280; Sotillos et al., 1997, Development 124:4769-4779; Wen et al., 1997, Development 124:4759-4767).

Adhesion assays have demonstrated that Notch-Delta interactions are physically mediated by the extracellular domains of the respective proteins (Fehon, et al., 1990, Cell 61:523-534). Furthermore deletion analyses have defined specific sequences that are responsible for this interaction (Rebay, et al., 1991, Cell 67:687-699; deletion mutants of Delta lacking the DSL domain fail to bind Notch (M. Muskavitch, personal communication); Fleming et al., 1997, Development 124:2973-2981). These assays were done under conditions where the Delta and the Notch proteins are overexpressed in S2 cells and full length Delta is clearly detected on the cell surface (data not shown). Interest was expressed to examine if Dl^{EC} is capable of binding to Notch.

Addition of Dl^{EC} to Notch expressing S2 cells followed by sedimentation through a sucrose cushion resulted in specific binding of Dl^{EC} to the Notch cells as compared to S2 cells alone (Figure 9A) suggesting a Dl^{EC} :Notch complex forms on these cells. These results were extended by analyzing the ability of Dl^{EC} to compete for full length Delta binding to Notch in a cell aggregation assay. In order to quantify the Notch/Delta interactions we have developed a turbidimetric assay which allows us to measure aggregation in a reproducible manner. Expression of Notch and Delta in S2 cells are induced for 16 hours with 0.085 mM and 0.022 mM $CuSO_4$, respectively. The cells are then centrifuged and raised in serum free media to an equivalent density yielding between 20-30% T_{320nm} ($\sim 2 \times 10^6$ cells/mL) in a Benchtop spectrophotometer. Blank values are set with M3 media alone.

400 μ L of Notch and 400 μ L of Delta cells are then pipeted into a 1.4 mL black sided, stoppered quartz cuvette and quickly inverted three times. The T_{320nm} is read immediately to determine the time "zero" value. The cuvette is then
5 rocked horizontally on a Thermolyne vari-mixer at 20 oscillations per minute and subsequent T_{320nm} readings are taken at one minute intervals. Change in T_{320nm} (relative to time zero) is then plotted versus time. The effect of Dl^{EC} was compared to a concentrate of media from ΔECN -S2 cells (closed squares) (Rebay et al., 1993, Cell 74:318-329)
10 prepared in parallel as these cells were transfected in the same manner with an irrelevant construct.

Pre-incubation of the Notch cells with Dl^{EC} concentrate resulted in a dramatic reduction in the initial rate of aggregation with Delta cells (Figure 9B). The
15 competitive effect of Dl^{EC} was sensitive to the concentration added and the time of preincubation with the Notch cells. Furthermore, pre-incubation of the Delta cells with Dl^{EC} had no effect on subsequent aggregation with Notch cells indicating Dl^{EC} specifically binds to Notch in a competitive
20 manner with respect to full length Delta.

The biological activity of Dl^{EC} was examined in a cell culture assay which was carried out as follows. Low density primary cultures of cortical neurons were prepared from embryonic day 15.5 to 16.5 mouse embryos. Single cell
25 suspensions in Dulbecco's modified Eagle medium high glucose/F12 (1:1), N2 Supplement, 2.5 mM L-glutamine and 5-10% fetal bovine serum were seeded on 5 mm diameter glass coverslips precoated with 15 μ g/ml poly-ornithine and 2 μ g/cm² laminin. After 10 days in culture, neurons (<1000/cm²) were growing on a monolayer of glial cells. To examine the
30 activity of Dl^{EC} , cultures were treated for 14-17 hours with a 1:10 dilution of either 5X ΔECN , 5X Dl^{EC} , purified Dl^{EC} (approximately 0.04 A_{280nm} /ml in 25 mM glycine, 30 mM Tris-HCl,

pH 7.0) made in culture medium. At least three independent culture wells were examined for each condition during one experimental trial. Cells were fixed in 4% paraformaldehyde, stained overnight with a mouse monoclonal antibody against neuron-specific class III β -tubulin (TuJ1, 1:500; BabCo, Berkeley, CA) and visualized with Cy3 conjugated anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories). Immunolabeled neurons were imaged with a Spot2 camera (Diagnostic Instruments) using a 40X objective on a Zeiss Axioplan 2 microscope and imported into Adobe Photoshop 4.0 (Adobe Systems, San Jose, CA). Neurite length was measured in five to ten randomly selected images from each coverslip using NIH Image 1.61 software and the data were analyzed with Sigma Plot 4.0 statistical software (SPSS).

Primary cultures of mouse cortical neurons expressing Notch endogenously develop dendritic processes (Figure 9C). It has been demonstrated that ligand-dependent Notch activation *in vitro* in cortical neurons expressing endogenous Notch receptors causes morphological changes and retraction of neurites. The same effects were observed when the neurons were cultured in the presence of enriched Dl^{EC} containing media or purified Dl^{EC} (Figure 9C). These data show that Dl^{EC} has biological activity consistent with the notion that it acts as an agonist. Similar effects of neurite outgrowth have also been observed with a soluble form of vertebrate Jagged (unpublished observation).

Amino acid sequence analysis was performed on the soluble Delta peptide. As described above, the molecular weight of Dl^{EC} estimated from SDS-PAGE analysis is consistent with Dl^{EC} being comprised of most if not all of the extracellular portion of the Delta protein. In addition, the N-terminal sequence of Dl^{EC} is consistent with the predicted N-terminus of full length Delta (Dl^{EC} is not proteolytically clipped at the N-terminus). Further, as described above, Dl^{EC}

likely arises due to proteolytic processing at a cleavage site(s) between the ninth EGF repeat and the transmembrane domain in a region designated the juxtamembrane domain. The sequence analysis was carried out by C-terminal sequencing
5 and by tryptic digest/liquid chromatography/mass spectrometry (LC/MS) of purified D1^{EC} derived from *Drosophila* Delta expressed in S2 cells. This analysis was carried out at the Harvard Microchemistry Facility, Cambridge MA.

The data generated by the C-terminal sequencing showed that the terminal residue was alanine. The amino acid
10 residue preceding the terminal residue showed heterogeneity with glycine being the most prevalent followed by asparagine, leucine, and arginine. These data indicate that D1^{EC} terminates at more than one position which indicates that more than one proteolytic processing site exists. However,
15 C-terminal sequencing is very difficult to perform and the confidence of residues beyond the terminal residue drops off significantly. However, analysis of the *Drosophila* Delta juxtamembrane domain (residues 564-594 of *Drosophila* Delta) shows four of six possible alanine residues that would give a
20 terminal sequence consistent with the C-terminal sequencing data, i.e., DA₅₇₆, GA₅₈₁, LA₅₉₁, and NA₅₉₃, (Figure 11). Our data indicated an alanine at position 591, in contrast to the sequence data of Vässin, et al., 1987, EMBO J. 6:3431-3440, which disclosed a threonine at that position.

The tryptic digest peptide analysis was consistent
25 with the C-terminal sequencing data. 24 tryptic digest peptides derived from *Drosophila* D1^{EC} were positively identified by LC/MS and their sequences determined. Five peptides were identified that terminated in the juxtamembrane domain. Two of the peptides terminated at residue Ala₅₉₃ and
30 two other peptides terminated at residue Ala₅₈₁. These data demonstrate that two prevalent forms of D1^{EC} terminate at amino acid residues 581 and 593. The fifth peptide

terminated at amino acid position Gln₅₇₈, which was not detected in the C-terminal analysis. The resolution of Ala₅₈₁ and Ala₅₉₃ by both analytical methods together indicates that the primary forms of Dl^{EC} are generated by cleavage at these
5 sites, although additional cleavage sites remain a possibility. The nature of these analyses do not permit a quantitative assessment of the relative proportion of the various species, thus it cannot be concluded which of the cleavage sites are preferred.

10 In conclusion, genetic and biochemical data show that Delta is cleaved to produce an active, functionally important extracellular fragment that is biologically active with an apparent agonistic function in the Notch pathway. Previous studies involving the *in vivo* expression of artificially truncated Notch ligands in *Drosophila* and other
15 systems have demonstrated both antagonistic and agonistic activities (Sun et al., 1997, Development 124:3439-3448; Fitzgerald et al., 1995, Development 121:4275-4282; Li et al., 1998, Immunity 8:43-55; Wang et al., 1998, Neuron 21:63-75). It is clear that soluble forms of Delta (DlS) can act
20 as antagonists in the developing *Drosophila* eye (Sun et al., 1997, Development 124:3439-3448). However, Dl^{EC} is not identical to DlS and therefore it is plausible that the two molecules may be functionally different. Figure 10 is a schematic comparing Dl^{EC} and DlS.

25 Although Kuz does not appear to be responsible for the constitutive cleavage of Notch, the possibility that Kuz can cleave Notch at alternative sites remains. In this regard, it has been claimed that KuzDN is able to inhibit transactivation of a target gene of the Notch pathway induced by ligand binding to the receptor (Logeat, et al., 1998,
30 Proc. Natl. Acad. Sci. USA 95:8108-8112). However it is possible that this effect does not reflect Notch cleavage but rather the cleavage of Delta to produce an active ligand.

Klueg et al., 1998, Mol. Cell Biol. 9:1709-1723 ("Klueg") have recently reported the processing of Delta during normal embryogenesis demonstrating the existence of Delta fragments, one of which is consistent with D1^{EC}. We note the
5 intermediate forms detected in the 16-20 hour embryos (Figures 7B, 8D, *kuz* +/-) are not present in *Kuz* mutants (Figure 8D, *kuz* -/-), raising the possibility that the generation of these products may also be mediated by *Kuz*.

The present invention is not to be limited in scope
10 by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

15 Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

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WHAT IS CLAIMED IS:

1. A peptide comprising a fragment of a Delta protein, the amino acid sequence of the peptide consisting of the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human
5 Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).
- 10 2. The peptide of claim 1 which comprises 30 contiguous amino acids of a Delta protein.
3. The peptide of claim 1 which comprises 100 contiguous amino acids of a Delta protein.
- 15 4. The peptide of claim 1 which comprises 150 contiguous amino acids of a Delta protein.
5. A purified derivative or analog of the peptide
20 of claim 1, which is able to display one or more functional activities of a Delta cleavage peptide.
6. A purified derivative or analog of the peptide of claim 1, which is able to display one or more functional
25 activities of a human or *D. melanogaster* Delta cleavage peptide.
7. The derivative or analog of claim 5 which is able to be bound by an antibody directed against a human or *D. melanogaster* Delta cleavage peptide.

30

8. A purified fragment of the peptide of claim 1, which is able to be bound by an antibody directed against a human Delta cleavage peptide.

5 9. A molecule comprising the fragment of claim 8.

10. A purified fragment of the peptide of claim 1 which is able to display one or more functional activities of a human Delta cleavage peptide.

10 11. A chimeric protein comprising a fragment of a Delta protein of not more than 200 amino acids, said fragment comprising the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick
15 Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9), fused via a covalent bond to an amino acid sequence of a second protein, in which the second protein is not the fragment of the Delta protein.

20 12. The chimeric protein of claim 11 in which the Delta cleavage peptide is of a human protein.

13. The chimeric protein of claim 12 which is able
25 to display one or more functional activities of a Delta cleavage peptide.

14. The peptide of claim 1 which is purified.

15. A fragment of a Delta protein of not more than
30 150 amino acids comprising a Delta sequence selected from the group consisting of amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃

in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

5

16. A fragment of a Delta protein of not more than 50 amino acids comprising a Delta sequence selected from the group consisting of amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in
10 chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

17. A fragment of a Delta protein of not more than
15 30 amino acids comprising a Delta sequence selected from the group consisting of amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in
20 *Xenopus* Delta (SEQ ID NO:8), and Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

18. A peptide the amino acid sequence consisting of amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human
25 Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

30

19. A molecule comprising the fragment of claim 15, 16 or 17 or the peptide of claim 18.

20. A chimeric protein comprising a Delta protein sequence fused to a non-Delta protein sequence, wherein the Delta protein sequence is a sequence of not more than 100 amino acids that comprises the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

10 21. A chimeric protein comprising a Delta protein sequence fused to a non-Delta protein sequence, wherein the Delta protein sequence is a sequence of not more than 50 amino acids that comprises the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

20 22. A chimeric protein comprising a Delta protein sequence fused to a non-Delta protein sequence, wherein the Delta protein sequence is a sequence of not more than 30 amino acids that comprises the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

30 23. A chimeric protein comprising a Delta protein sequence fused to a non-Delta protein sequence wherein the Delta sequence is selected from the group consisting of amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ

ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), and Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

24. The fragment or peptide of claim 15, 16, 17 or 18, which is purified.

25. An antibody, or a fragment thereof, directed against the Delta sequence of the fragment or peptide of claim 15, 16, 17 or 18 or the chimeric protein of claim 20, 21 or 22.

26. A method of modulating activity of Notch or Delta or Kuz or at least one of their signalling pathways in a cell, or organism comprising a cell, that expresses Notch or Delta or Kuz comprising contacting the cell or organism with the fragment of claim 15, 16 or 17 or peptide or molecule of claim 18 or 19 or protein of claim 20, 21 or 22 or the fragment or peptide of claim 24.

27. A method of modulating activity of Notch or Delta or Kuz or at least one of their signalling pathways in a cell or organism that expresses Notch or Delta or Kuz comprising contacting the cell or organism with the antibody of claim 25.

28. A method of modulating activity of Notch or Delta or Kuz or at least one of their signalling pathways in a cell or organism that expresses Notch or Delta or Kuz comprising recombinantly expressing within the cell or organism the fragment of claim 15, 16 or 17 or peptide or

molecule of claim 18 or 19 or protein of claim 20, 21 or 22 or the fragment or peptide of claim 24.

29. A method of modulating activity of Notch or
5 Delta or Kuz or at least one of their signalling pathways in a cell or organism that expresses Notch or Delta or Kuz comprising recombinantly expressing within the cell or organism the antibody of claim 25.

30. The fragment of claim 15, 16 or 17 or the
10 peptide of claim 18, which is amino- or carboxy-terminal derivatized.

31. The fragment or peptide of claim 30 which is
N-acetylated.
15

32. The fragment or peptide of claim 30 which has a C-terminal amide.

33. A kit comprising in a container the fragment
20 of claim 15, 16 or 17 or the peptide of claim 18.

34. A pharmaceutical composition comprising the fragment of claim 15, 16 or 17 or the peptide of claim 18, in purified form; and a pharmaceutically acceptable carrier.

25 35. A transgenic non-human animal containing a transgene encoding the fragment of claim 15, 16 or 17 or the peptide of claim 18 or the protein of claim 20, 21 or 22.

30 36. A nucleic acid comprising a nucleotide sequence encoding a fragment of Delta of not more than 200 amino acids, said fragment comprising the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅

to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

5

37. The nucleic acid of claim 36 which is isolated.

38. The nucleic acid of claim 36 which is DNA.

10

39. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 36.

40. A recombinant cell containing the nucleic acid
15 of claim 36.

41. A method of producing a Delta cleavage peptide comprising growing a recombinant cell containing the nucleic acid of claim 36 such that the encoded Delta cleavage peptide
20 is expressed by the cell, and recovering the expressed Delta cleavage peptide.

42. The product of the process of claim 41.

25 43. A pharmaceutical composition comprising a therapeutically effective amount of a fragment of a Delta protein, the amino acid sequence of the fragment consisting of the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta
30 (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila*

Delta (SEQ ID NO:9); and a pharmaceutically acceptable carrier.

44. The composition of claim 43 in which the Delta
5 protein is a human Delta protein.

45. A pharmaceutical composition comprising a
therapeutically effective amount of a derivative or analog of
a Delta cleavage peptide, which derivative or analog is
characterized by the ability to bind to a Kuz protein; and a
10 pharmaceutically acceptable carrier.

46. A pharmaceutical composition comprising a
therapeutically effective amount of the nucleic acid of claim
36; and a pharmaceutically acceptable carrier.
15

47. A pharmaceutical composition comprising a
therapeutically effective amount of an antibody which binds
to a Delta cleavage peptide and a pharmaceutically acceptable
carrier.

20 48. A pharmaceutical composition comprising a
therapeutically effective amount of a fragment or derivative
of an antibody to a Delta cleavage peptide containing the
binding domain of the antibody; and a pharmaceutically
acceptable carrier.
25

49. A method of treating or preventing a disease
or disorder in a subject comprising administering to a
subject in which such treatment or prevention is desired a
therapeutically effective amount of a fragment of a Delta
30 protein, the amino acid sequence of the fragment consisting
of the amino acid sequence Cys₅₁₆ to amino acid Phe₅₄₃ in human
Delta (SEQ ID NO:10), Cys₅₁₅ to amino acid Phe₅₄₃ in mouse

Delta (SEQ ID NO:6), Cys₅₂₃ to amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7), Cys₅₁₈ to amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8), or Cys₅₆₄ to amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9), or a derivative of any of the foregoing
5 which is able to bind to a Kuz protein.

50. The method according to claim 49 in which the disease or disorder is a malignancy characterized by increased Delta activity or increased expression of a Delta protein or of a Delta derivative capable of being bound by an
10 anti-Delta antibody, relative to said Delta activity or expression in an analogous non-malignant sample.

51. The method according to claim 50 in which the disease or disorder is selected from the group consisting of
15 cervical cancer, breast cancer, colon cancer, melanoma, seminoma, and lung cancer.

52. The method according to claim 50 in which the subject is a human.
20

53. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired an effective amount of the nucleic acid of claim 36.

25 54. The method according to claim 50 in which the disease or disorder is a disease or disorder of the central nervous system.

55. A method of diagnosing a disease or disorder
30 characterized by an aberrant level of Notch-Delta protein binding activity in a patient, comprising measuring the ability of a Delta cleavage peptide in a sample derived from

the patient to bind to a Kuz protein, in which an increase or decrease in the ability of the peptide to bind to the Kuz protein, relative to the ability found in an analogous sample from a normal individual, indicates the presence of the
5 disease or disorder in the patient.

56. A purified complex of a Delta protein and a Kuz protein.

10 57. The purified complex of claim 56 in which the proteins are human proteins.

58. A purified complex selected from the group consisting of a complex of a derivative of Delta and Kuz, a complex of Delta and a derivative of Kuz, and a complex of a
15 derivative of Delta and a derivative of Kuz; in which the derivative of Delta is able to form a complex with a wild-type Kuz and the derivative of Kuz is able to form a complex with wild-type Delta.

20 59. The purified complex of claim 58 in which the derivative of Delta or Kuz is fluorescently labeled.

60. A chimeric protein comprising a fragment of Delta consisting of at least 6 amino acids fused via a covalent bond to a fragment of Kuz consisting of at least 6
25 amino acids.

61. The chimeric protein of claim 60 in which the fragment of Delta is a fragment capable of binding Kuz and in which the fragment of Kuz is a fragment capable of binding
30 Delta.

62. The chimeric protein of claim 61 in which the fragment of Delta and the fragment of Kuz form a Delta:Kuz complex.

5 63. An antibody which immunospecifically binds the complex of claim 58 or a fragment or derivative of said antibody containing the binding domain thereof.

10 64. The antibody of claim 63 which does not immunospecifically bind Delta or Kuz that is not part of a Delta:Kuz complex.

15 65. An isolated nucleic acid or an isolated combination of nucleic acids comprising a nucleotide sequence encoding a Delta protein and a nucleotide sequence encoding a Kuz protein.

20 66. The isolated nucleic acid or isolated combination of nucleic acids of claim 65 which are nucleic acid vectors.

25 67. The isolated nucleic acid or isolated combination of nucleic acids of claim 65 in which the Delta coding sequence and the Kuz coding sequence are operably linked to a promoter.

30 68. An isolated nucleic acid that comprises a nucleotide sequence encoding the chimeric protein of claim 60.

30 69. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the complex of claim 56; and a pharmaceutically acceptable carrier.

70. The pharmaceutical composition of claim 70 in which the proteins are human proteins.

71. A method of producing a complex of Delta and Kuz comprising growing a recombinant cell containing the nucleic acid of claim 65 such that the encoded Delta and Kuz proteins are expressed and bind to each other, and recovering the expressed complex of Delta and Kuz.

72. A method of diagnosing or screening for the presence of or a predisposition for developing a disease or disorder characterized by an aberrant level of a complex of Delta and Kuz, in a subject comprising measuring the level of said complex, RNA encoding Delta and Kuz, or functional activity of said complex in a sample derived from the subject, in which an increase or decrease in the level of said complex, said RNA encoding Delta and Kuz, or functional activity of said complex in the sample, relative to the level of said complex, said RNA encoding Delta and Kuz or functional activity of said complex found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or disorder, indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.

73. A kit comprising in one or more containers a substance selected from the group consisting of a complex of Delta and Kuz, an antibody against said complex, nucleic acid probes capable of hybridizing to RNA of Delta and RNA of Kuz, or pairs of nucleic acid primers capable of priming amplification of at least a portion of the Delta gene and the Kuz gene.

74. A method of treating or preventing a disease or disorder involving aberrant levels of a complex of Delta and Kuz, in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule or molecules that modulate the function of said complex.

75. A method of screening for a molecule that modulates directly or indirectly the formation of a complex of Delta and Kuz comprising measuring the levels of said complex formed from Delta and Kuz proteins in the presence of said molecule under conditions conducive to formation of the complex; and comparing the levels of said complex with the levels of said complex that are formed in the absence of said molecule, wherein a lower or higher level of said complex in the presence of said molecule indicates that the molecule modulates formation of said complex.

76. A recombinant non-human animal in which both an endogenous *Delta* gene and an endogenous *Kuz* have been deleted or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof.

77. A recombinant non-human animal containing both a *Delta* gene and a *Kuz* gene, in which the *Delta* gene is under the control of a promoter that is not the native *Delta* gene promoter and the *Kuz* gene is under the control of a promoter that is not the native *Kuz* gene promoter.

78. A method of modulating the activity or levels of Delta by contacting a cell with, or administering an animal expressing a *Delta* gene, a *Kuz* protein, or a nucleic

acid encoding said protein or an antibody that immunospecifically binds said protein or a fragment or derivative of said antibody containing the binding domain thereof.

5

79. A method of modulating the activity or levels of Kuz by contacting a cell with, or administering an animal expressing a gene encoding said protein, Delta, or a nucleic acid encoding Delta, or an antibody that immunospecifically binds Delta or a fragment or derivative of said antibody
10 containing the binding domain thereof.

80. A method for identifying a molecule that modulates activity of Delta or Kuz or a complex of Delta and Kuz comprising contacting one or more candidate molecules
15 with Delta in the presence of Kuz; and measuring the amount of complex that forms between Delta and Kuz; wherein an increase or decrease in the amount of complex that forms relative to the amount that forms in the absence of the candidate molecules indicates that the molecules modulate the
20 activity of Delta or Kuz or said complex of Delta and Kuz.

81. A method for detecting or measuring Delta activation in a cell comprising detecting or measuring the expression of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, wherein the
25 presence and amount of Delta cleavage products indicates the presence and amount, respectively, of Delta activation.

82. The method according to claim 81 in which said detecting or measuring is carried out by a method comprising
30 contacting a cell with a molecule that binds to D1^{EC} or D1TM under conditions conducive to specific binding; and detecting any binding of the molecule to the cell that occurs.

83. The method according to claim 82 in which the molecule is an anti-Delta antibody or a binding region thereof.

5 84. The method according to claim 82 in which the molecule is Notch or Kuz or a binding region thereof.

85. The method according to claim 83 in which the antibody or binding region thereof is labelled with a fluorescent label, and binding of the antibody to the cell is
10 detected or measured by fluorescent activated cell sorting.

86. The method according to claim 81 in which said detecting or measuring is carried out by a method comprising (a) contacting the cell with a reagent that binds to or
15 reacts with cell surface proteins under conditions conducive to such binding or reaction; and (b) detecting any such binding to or reaction with Delta.

87. The method according to claim 86 in which the
20 reagent is labeled.

88. The method according to claim 86 in which said detecting is carried out by a method comprising contacting the cell with a labeled specific binding partner to the reagent.
25

89. The method according to claim 87 or 88 in which the detecting of any such binding or reaction in step (b) is carried out by western blotting or immunoprecipitation, using an anti-Delta antibody.
30

90. A method for detecting or measuring Delta activation in a cell comprising detecting or measuring an

amino-terminal fragment of Delta terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ in *Drosophila* Delta, between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta, between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta, between
5 amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta, or terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta.

91. A method for detecting or measuring Delta
10 activation in a cell comprising detecting or measuring under reducing conditions, a soluble Delta fragment of approximately 67 kilodaltons.

92. A method for detecting or measuring Kuz
15 function in a cell comprising detecting or measuring the expression of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, wherein the presence and amount of Delta cleavage products indicates the presence and amount, respectively, of Kuz function.

20 93. The method according to claim 92 in which said detecting or measuring is carried out by a method comprising contacting a cell with a molecule that binds to D1^{EC} or D1TM under conditions conducive to specific binding; and detecting any binding of the molecule to the cell that occurs.

25 94. The method according to claim 93 in which the molecule is an anti-Delta antibody or a binding region thereof.

30 95. A method for detecting or measuring Kuz function in a cell comprising detecting or measuring an amino-terminal fragment of a Delta protein terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ in *Drosophila*

Delta, between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human
Delta, between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse
Delta, between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick
Delta, or terminating between amino acid Cys₅₁₈ and amino acid
5 Phe₅₄₄ in *Xenopus* Delta.

96. A method for detecting or measuring Kuz
function in a cell comprising detecting or measuring under
reducing conditions, a soluble Delta fragment of
10 approximately 67 kilodaltons.

97. A method for identifying a modulator of Delta
activation comprising providing a cell with a candidate
modulator molecule and detecting or measuring the expression
by the cell of one or more Delta cleavage products selected
15 from the group consisting of D1^{EC} and D1TM, in which a
difference in the presence or amount of said one or more
cleavage products compared to a Delta cell not contacted with
the candidate molecule indicates that the molecule modulates
Delta activity.

20

98. A method for identifying a modulator of Delta
activation comprising contacting a cell with a candidate
modulator molecule and detecting or measuring the amount of
the expression an amino-terminal fragment of a Delta protein
terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ in
25 *Drosophila* Delta, between amino acid Cys₅₁₆ and amino acid
Phe₅₄₃ in human Delta, between amino acid Cys₅₁₅ and amino acid
Phe₅₄₃ in mouse Delta, between amino acid Cys₅₂₃ and amino acid
Phe₅₅₁ in chick Delta, or terminating between amino acid Cys₅₁₈
and amino acid Phe₅₄₄ in *Xenopus* Delta.; in which a difference
30 in the presence or amount of said fragment compared to a
Delta cell not contacted with the candidate molecule
indicates that the molecule modulates Delta activity.

99. A method for identifying a modulator of Delta activation comprising contacting a cell with a candidate modulator molecule and detecting or measuring under reducing conditions, a soluble Delta fragment of approximately 67
5 kilodaltons, in which a difference in the presence or amount of said soluble Delta fragment compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Delta activity.

100. A method for identifying a modulator of Kuz
10 function comprising providing a cell with a candidate modulator molecule and detecting or measuring the expression by the cell of one or more Delta cleavage products selected from the group consisting of D1^{EC} and D1TM, in which a difference in the presence or amount of said one or more
15 cleavage products compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

101. A method for identifying a modulator of Kuz
20 function comprising contacting a cell with a candidate modulator molecule and detecting or measuring the amount of the expression an amino-terminal fragment of a Delta protein terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ in *Drosophila* Delta, between amino acid Cys₅₁₆ and amino acid
25 Phe₅₄₃ in human Delta, between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta, between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta, or terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta.; in which a difference
30 in the presence or amount of said fragment compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

102. A method for identifying a modulator of Kuz function comprising contacting a cell with a candidate modulator molecule and detecting or measuring under reducing conditions, a soluble Delta fragment of approximately 67
5 kilodaltons, in which a difference in the presence or amount of said soluble Delta fragment compared to a Delta cell not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

103. A method for identifying a modulator of Delta
10 activation comprising contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and optionally a composition comprising cellular proteins, under conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition
15 and detecting or measuring the amount of Delta cleavage products D1^{EC} and D1TM that result, in which a difference in the presence or amount of said Delta cleavage products compared to a full-length Delta in presence of said composition not contacted with the candidate molecule
20 indicates that the molecule modulates Delta activity.

104. The method according to claim 103 in which the composition is a cell lysate made from cells which recombinantly express Delta.

25 105. The method according to claim 103 in which the composition is a cell lysate made from cells which endogenously express Delta.

106. A method for identifying a modulator of Kuz
30 function comprising contacting a candidate modulator molecule with a full length Delta in the presence of Kuz and optionally a composition comprising cellular proteins, under

conditions conducive to cleavage of the full-length Delta by Kuz and optionally one or more components of the composition and detecting or measuring the amount of Delta cleavage products Dl^{EC} and Dl^{TM} that result, in which a difference in
5 the presence or amount of said Delta cleavage products compared to a full-length Delta in presence of said composition not contacted with the candidate molecule indicates that the molecule modulates Kuz function.

10 107. The method according to claim 106 in which the composition is a cell lysate made from cells which recombinantly express Kuz.

15 108. The method according to claim 106 in which the composition is a cell lysate made from cells which endogenously express Kuz.

20 109. A purified fragment of a Delta protein, the sequence of said fragment consisting of the amino acid sequence beginning at amino acid Ser_{22} and terminating between amino acid Cys_{516} and amino acid Phe_{543} in human Delta (SEQ ID NO:10); beginning at amino acid Ser_{22} and terminating between amino acid Cys_{515} and amino acid Phe_{543} in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser_{24} and terminating between amino acid Cys_{523} and amino acid Phe_{551} in chick Delta (SEQ ID
25 NO:7); beginning at amino acid Ser_{22} and terminating between amino acid Cys_{518} and amino acid Phe_{544} in *Xenopus* Delta (SEQ ID NO:8); and the sequence beginning at amino acid Ser_{23} and terminating between amino acid Cys_{564} and amino acid Ala_{593} in *Drosophila* Delta (SEQ ID NO:9).

30 110. The fragment of claim 109, which is amino- or carboxy-terminal derivatized.

111. The fragment of claim 110, which is N-acetylated.

112. The fragment of claim 110, which has a C-terminal amide.

113. A nucleic acid comprising a nucleotide sequence encoding a fragment of a Delta protein, the amino acid sequence of said fragment consisting of an amino acid sequence beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₆ and amino acid Phe₅₄₃ in human Delta (SEQ ID NO:10); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₅ and amino acid Phe₅₄₃ in mouse Delta (SEQ ID NO:6); beginning at amino acid Ser₂₄ and terminating between amino acid Cys₅₂₃ and amino acid Phe₅₅₁ in chick Delta (SEQ ID NO:7); beginning at amino acid Ser₂₂ and terminating between amino acid Cys₅₁₈ and amino acid Phe₅₄₄ in *Xenopus* Delta (SEQ ID NO:8); and the sequence beginning at amino acid Ser₂₃ and terminating between amino acid Cys₅₆₄ and amino acid Ala₅₉₃ in *Drosophila* Delta (SEQ ID NO:9).

114. The nucleic acid of claim 113 which is isolated.

115. The nucleic acid of claim 113 which is DNA.

116. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence encoding a fragment of Delta of nucleic acid of claim 113.

117. A peptide comprising a fragment of a Delta protein, the amino acid sequence of the fragment consisting of the amino acid sequence Cys₅₆₄ to amino acid Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

118. A chimeric protein comprising a fragment of a Delta protein of not more than 200 amino acids, said fragment comprising the amino acid sequence Cys₅₆₄ to amino acid Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9), fused via a covalent bond
5 to an amino acid sequence of a second protein, in which the second protein is not the fragment of the Delta protein.

119. A peptide the amino acid sequence of which consists of amino acid sequence Cys₅₆₄ to amino acid Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).
10

120. A nucleic acid comprising a nucleotide sequence encoding a fragment of a Delta protein of not more than 200 amino acids, said fragment comprising the amino acid sequence Cys₅₆₄ to amino acid Gln₅₉₄ in *Drosophila* Delta (SEQ
15 ID NO:9).

121. A method for detecting or measuring Delta activation in a cell comprising detecting or measuring an amino-terminal fragment of Delta terminating between amino
20 acid Cys₅₆₄ and amino acid Gln₅₉₄ in *Drosophila* Delta.

122. A method for detecting or measuring Kuz function in a cell comprising detecting or measuring an amino-terminal fragment of a Delta protein terminating
25 between amino acid Cys₅₆₄ and amino acid Gln₅₉₄ in *Drosophila* Delta.

123. A purified fragment of a Delta protein, the amino acid sequence of said fragment consisting of the amino acid sequence beginning at amino acid Ser₂₃ and terminating
30 between amino acid Cys₅₆₄ and amino acid Gln₅₉₄ in *Drosophila* Delta (SEQ ID NO:9).

124. A nucleic acid comprising a nucleotide
sequence encoding a fragment of a Delta protein, the amino
acid sequence of said fragment consisting of an amino acid
sequence beginning at amino acid Ser₂₃ and terminating between
5 amino acid Cys₅₆₄ and amino acid Gln₅₉₄ in *Drosophila* Delta
(SEQ ID NO:9).

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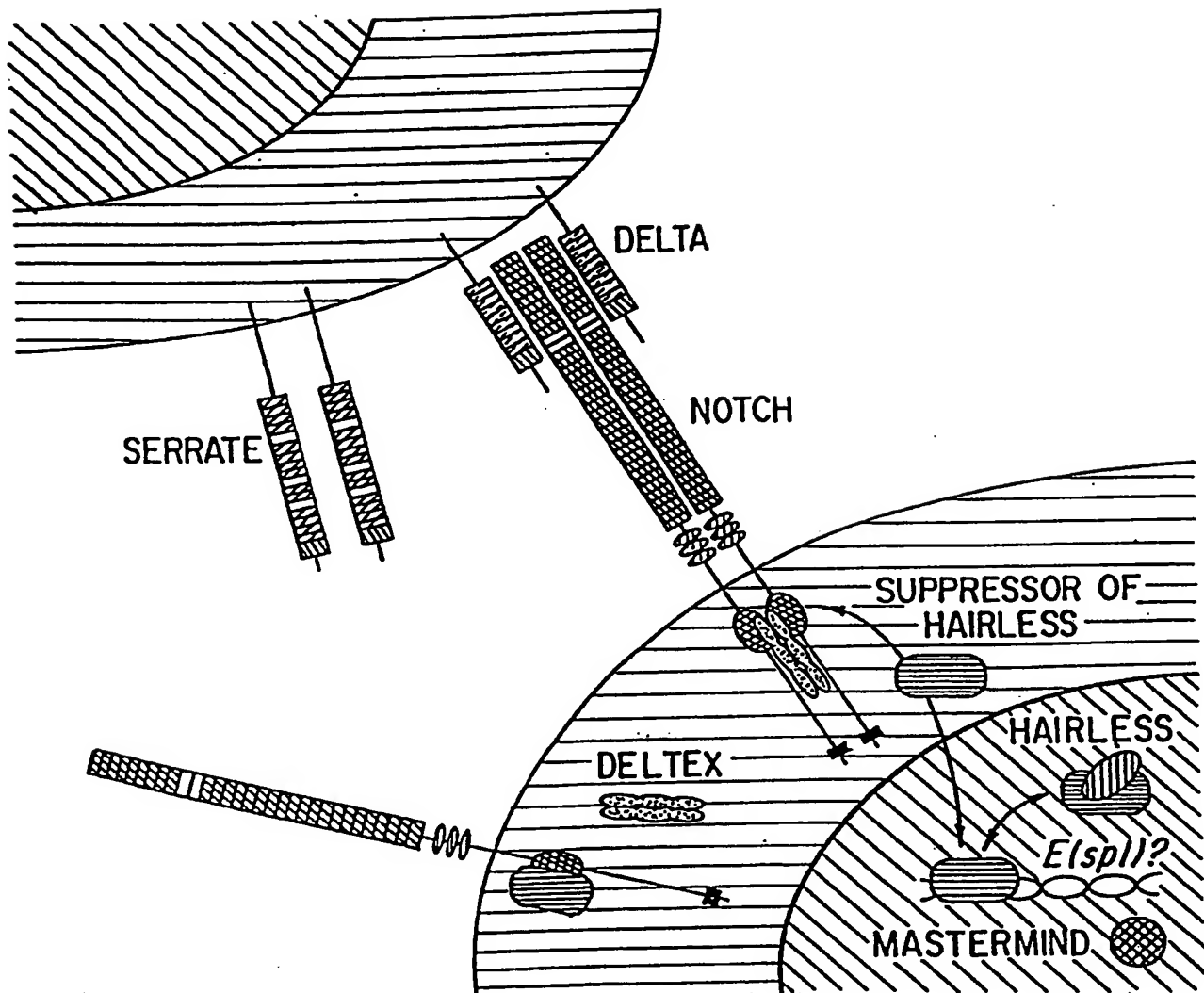


FIGURE 1

Alignment of Notch Homologs

Partial Repeat Change Sites		Key Sites		
10000	10001	10002	10003	10004
10005	10006	10007	10008	10009
10010	10011	10012	10013	10014
10015	10016	10017	10018	10019
10020	10021	10022	10023	10024
10025	10026	10027	10028	10029
10030	10031	10032	10033	10034
10035	10036	10037	10038	10039
10040	10041	10042	10043	10044
10045	10046	10047	10048	10049
10050	10051	10052	10053	10054
10055	10056	10057	10058	10059
10060	10061	10062	10063	10064
10065	10066	10067	10068	10069
10070	10071	10072	10073	10074
10075	10076	10077	10078	10079
10080	10081	10082	10083	10084
10085	10086	10087	10088	10089
10090	10091	10092	10093	10094
10095	10096	10097	10098	10099
10100	10101	10102	10103	10104
10105	10106	10107	10108	10109
10110	10111	10112	10113	10114
10115	10116	10117	10118	10119
10120	10121	10122	10123	10124
10125	10126	10127	10128	10129
10130	10131	10132	10133	10134
10135	10136	10137	10138	10139
10140	10141	10142	10143	10144
10145	10146	10147	10148	10149
10150	10151	10152	10153	10154
10155	10156	10157	10158	10159
10160	10161	10162	10163	10164
10165	10166	10167	10168	10169
10170	10171	10172	10173	10174
10175	10176	10177	10178	10179
10180	10181	10182	10183	10184
10185	10186	10187	10188	10189
10190	10191	10192	10193	10194
10195	10196	10197	10198	10199
10200	10201	10202	10203	10204
10205	10206	10207	10208	10209
10210	10211	10212	10213	10214
10215	10216	10217	10218	10219
10220	10221	10222	10223	10224
10225	10226	10227	10228	10229
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10235	10236	10237	10238	10239
10240	10241	10242	10243	10244
10245	10246	10247	10248	10249
10250	10251	10252	10253	10254
10255	10256	10257	10258	10259
10260	10261	10262	10263	10264
10265	10266	10267	10268	10269
10270	10271	10272	10273	10274
10275	10276	10277	10278	10279
10280	10281	10282	10283	10284
10285	10286	10287	10288	10289
10290	10291	10292	10293	10294
10295	10296	10297	10298	10299
10300	10301	10302	10303	10304
10305	10306	10307	10308	10309
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10315	10316	10317	10318	10319
10320	10321	10322	10323	10324
10325	10326	10327	10328	10329
10330	10331	10332	10333	10334
10335	10336	10337	10338	10339
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10345	10346	10347	10348	10349
10350	10351	10352	10353	10354
10355	10356	10357	10358	10359
10360	10361	10362	10363	10364
10365	10366	10367	10368	10369
10370	10371	10372	10373	10374
10375	10376	10377	10378	10379
10380	10381	10382	10383	10384
10385	10386	10387	10388	10389
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10395	10396	10397	10398	10399
10400	10401	10402	10403	10404
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10410	10411	10412	10413	10414
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10420	10421	10422	10423	10424
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10430	10431	10432	10433	10434
10435	10436	10437	10438	10439
10440	10441	10442	10443	10444
10445	10446	10447	10448	10449
10450	10451	10452	10453	10454
10455	10456	10457	10458	10459
10460	10461	10462	10463	10464
10465	10466	10467	10468	10469
10470	10471	10472	10473	10474
10475	10476	10477	10478	10479
10480	10481	10482	10483	10484
10485	10486	10487	10488	10489
10490	10491	10492	10493	10494
10495	10496	10497	10498	10499
10500	10501	10502	10503	10504
10505	10506	10507	10508	10509
10510	10511	10512	10513	10514
10515	10516	10517	10518	10519
10520	10521	10522	10523	10524
10525	10526	10527	10528	10529
10530	10531	10532	10533	10534
10535	10536	10537	10538	10539
10540	10541	10542	10543	10544
10545	10546	10547	10548	10549
10550	10551	10552	10553	10554
10555	10556	10557	10558	10559
10560	10561	10562	10563	10564
10565	10566	10567	10568	10569
10570	10571	10572	10573	10574
10575	10576	10577	10578	10579
10580	10581	10582	10583	10584
10585	10586	10587	10588	10589
10590	10591	10592	10593	10594
10595	10596	10597	10598	10599
10600	10601	10602	10603	10604
10605	10606	10607	10608	10609
10610	10611	10612	10613	10614
10615	10616	10617	10618	10619
10620	10621	10622	10623	10624
10625	10626	10627	10628	10629
10630	10631	10632	10633	10634
10635	10636	10637	10638	10639
10640	10641	10642	10643	10644
10645	10646	10647	10648	10649
10650	10651	10652	10653	10654
10655	10656	10657	10658	10659
10660	10661	10662	10663	10664
10665	10666	10667	10668	10669
10670	10671	10672	10673	10674
10675	10676	10677	10678	10679
10680	10681	10682	10683	10684
10685	10686	10687	10688	10689
10690	10691	10692	10693	10694
10695	10696	10697	10698	10699
10700	10701	10702	10703	10704
10705	10706	10707	10708	10709
10710	10711	10712	10713	10714
10715	10716	10717	10718	10719
10720	10721	10722	10723	10724
10725	10726	10727	10728	10729
10730	10731	10732	10733	10734
10735	10736	10737	10738	10739
10740	10741	10742	10743	10744
10745	10746	10747	10748	10749
10750	10751	10752	10753	10754
10755	10756	10757	10758	10759
10760	10761	10762	10763	10764
10765	10766	10767	10768	10769
10770	10771	10772	10773	10774
10775	10776	10777	10778	10779
10780	10781	10782	10783	10784
10785	10786	10787	10788	10789
10790	10791	10792	10793	10794
10795	10796	10797	10798	10799
10800	10801	10802	10803	10804
10805	10806	10807	10808	10809
10810	10811	10812	10813	10814
10815	10816	10817	10818	10819
10820	10821	10822	10823	10824
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10830	10831	10832	10833	10834
10835	10836	10837	10838	10839
10840	10841	10842	10843	10844
10845	10846	10847	10848	10849
10850	10851	10852	10853	10854
10855	10856	10857	10858	10859
10860	10861	10862	10863	10864
10865	10866	10867	10868	10869
10870	10871	10872	10873	10874
10875	10876	10877	10878	10879
10880	10881	10882	10883	10884
10885	10886	10887	10888	10889
10890	10891	10892	10893	10894
10895	10896	10897	10898	10899
10900	10901	10902	10903	10904
10905	10906	10907	10908	10909
10910	10911	10912	10913	10914
10915	10916	10917	10918	10919
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10935	10936	10937	10938	10939
10940	10941	10942	10943	10944
10945	10946	10947	10948	10949
10950	10951	10952	10953	10954
10955	10956	10957	10958	10959
10960	10961	10962	10963	10964
10965	10966	10967	10968	10969
10970	10971	10972	10973	10974
10975	10976	10977	10978	10979
10980	10981	10982	10983	10984
10985	10986	10987	10988	10989
10990	10991	10992	10993	10994
10995	10996	10997	10998	10999
11000	11001	11002	11003	11004
11005	11006	11007	11008	11009
11010	11011	11012	11013	11014
11015	11016	11017	11018	11019
11020	11021	11022	11023	11024
11025	11026	11027	11028	11029
11030	11031	11032	11033	11034
11035	11036	11037	11038	11039
11040	11041	11042	11043	11044
11045	11046	11047	11048	11049
11050	11051	11052	11053	11054
11055	11056	11057	11058	11059
11060	11061	11062	11063	11064
11065	11066	11067	11068	11069
11070	11071	11072	11073	11074
11075	11076	11077	11078	11079

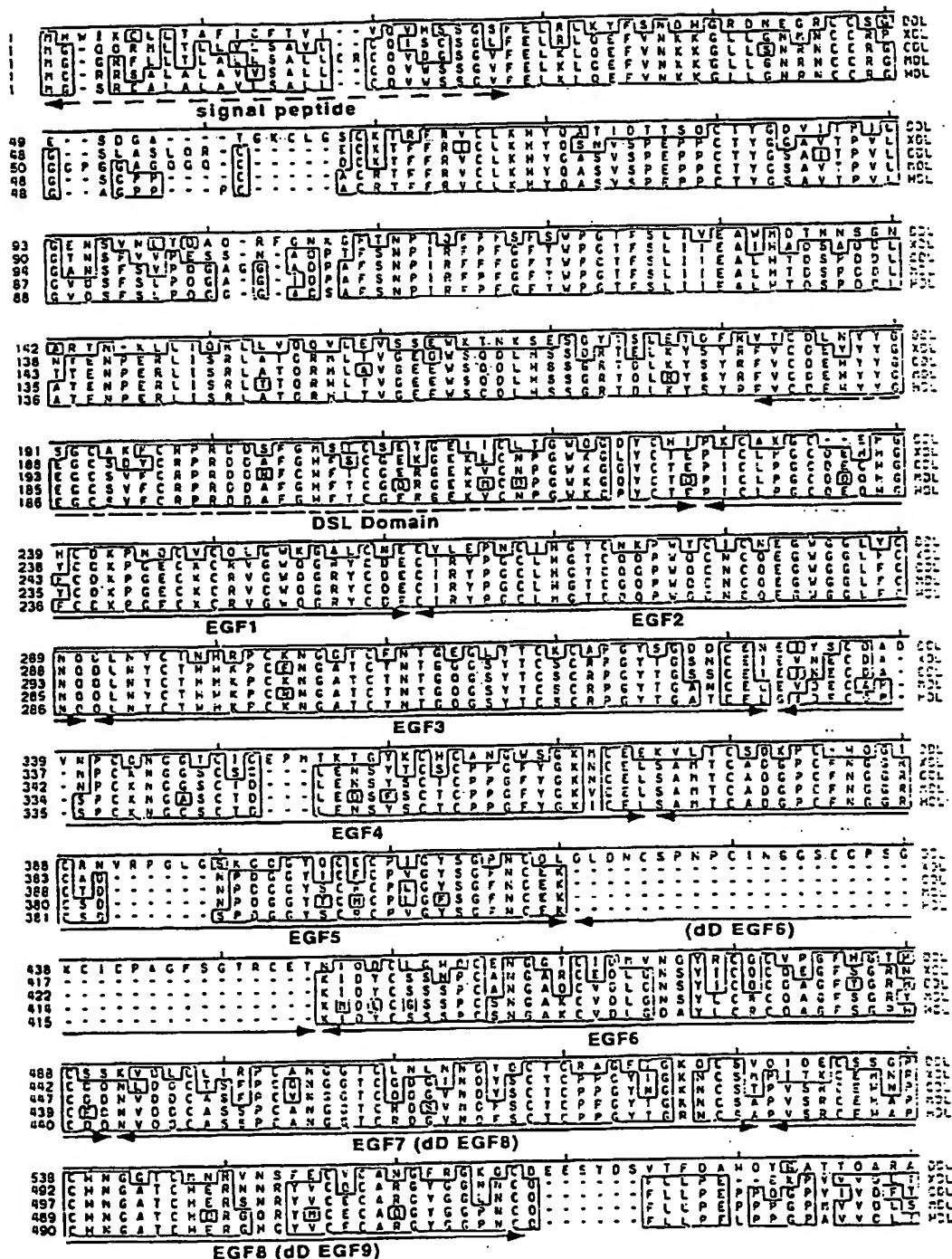


FIGURE 3

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Met	Gly	Ser	Arg	Cys	Ala	Leu	Ala	Leu	Ala	Val	Leu	Ser	Ala	Leu	Leu
1				5					10					15	
Cys	Gln	Val	Trp	Ser	Ser	Gly	Val	Phe	Glu	Leu	Lys	Leu	Gln	Glu	Phe
			20					25					30		
Val	Asn	Lys	Lys	Gly	Leu	Leu	Gly	Asn	Arg	Asn	Cys	Cys	Arg	Gly	Gly
		35					40					45			
Ala	Gly	Pro	Pro	Pro	Cys	Ala	Cys	Arg	Thr	Phe	Phe	Arg	Val	Cys	Leu
	50					55					60				
Lys	His	Tyr	Gln	Ala	Ser	Val	Ser	Pro	Glu	Pro	Pro	Cys	Thr	Tyr	Gly
65					70					75				80	
Ser	Ala	Val	Thr	Pro	Val	Leu	Gly	Val	Asp	Ser	Phe	Ser	Leu	Pro	Asp
				85					90					95	
Gly	Gly	Gly	Ala	Asp	Ser	Ala	Phe	Ser	Asn	Pro	Ile	Arg	Phe	Pro	Phe
			100					105					110		
Gly	Phe	Thr	Trp	Pro	Gly	Thr	Phe	Ser	Leu	Ile	Ile	Glu	Ala	Leu	His
	115						120					125			
Thr	Asp	Ser	Pro	Asp	Asp	Leu	Ala	Thr	Glu	Asn	Pro	Glu	Arg	Leu	Ile
	130					135					140				
Ser	Arg	Leu	Ala	Thr	Gln	Arg	His	Leu	Thr	Val	Gly	Glu	Glu	Trp	Ser
145					150					155				160	
Gln	Asp	Leu	His	Ser	Ser	Gly	Arg	Thr	Asp	Leu	Lys	Tyr	Ser	Tyr	Arg
				165					170					175	
Phe	Val	Cys	Asp	Glu	His	Tyr	Tyr	Gly	Glu	Gly	Cys	Ser	Val	Phe	Cys
			180					185					190		
Arg	Pro	Arg	Asp	Asp	Ala	Phe	Gly	His	Phe	Thr	Cys	Gly	Glu	Arg	Gly
	195						200					205			
Glu	Lys	Val	Cys	Asn	Pro	Gly	Trp	Lys	Gly	Pro	Tyr	Cys	Thr	Glu	Pro
	210					215					220				
Ile	Cys	Leu	Pro	Gly	Cys	Asp	Glu	Gln	His	Gly	Phe	Cys	Asp	Lys	Pro
225					230					235				240	
Gly	Glu	Cys	Lys	Cys	Arg	Val	Gly	Trp	Gln	Gly	Arg	Tyr	Cys	Asp	Glu
				245					250					255	
Cys	Ile	Arg	Tyr	Pro	Gly	Cys	Leu	His	Gly	Thr	Cys	Gln	Gln	Pro	Trp
			260					265					270		
Gln	Cys	Asn	Cys	Gln	Glu	Gly	Trp	Gly	Gly	Leu	Phe	Cys	Asn	Gln	Asp
		275					280					285			
Leu	Asn	Tyr	Cys	Thr	His	His	Lys	Pro	Cys	Lys	Asn	Gly	Ala	Thr	Cys
	290					295					300				
Thr	Asn	Thr	Gly	Gln	Gly	Ser	Tyr	Thr	Cys	Ser	Cys	Arg	Pro	Gly	Tyr
305					310					315				320	
Thr	Gly	Ala	Thr	Cys	Glu	Leu	Gly	Ile	Asp	Glu	Cys	Asp	Pro	Ser	Pro
				325					330					335	
Cys	Lys	Asn	Gly	Gly	Ser	Cys	Thr	Asp	Leu	Glu	Asn	Ser	Tyr	Ser	Cys
			340					345					350		
Thr	Cys	Pro	Pro	Gly	Phe	Tyr	Gly	Lys	Ile	Cys	Glu	Leu	Ser	Ala	Met
		355					360					365			
Thr	Cys	Ala	Asp	Gly	Pro	Cys	Phe	Asn	Gly	Gly	Arg	Cys	Ser	Asp	Ser
	370					375					380				
Pro	Asp	Gly	Gly	Tyr	Ser	Cys	Arg	Cys	Pro	Val	Gly	Tyr	Ser	Gly	Phe
385					390					395				400	
Asn	Cys	Glu	Lys	Lys	Ile	Asp	Tyr	Cys	Ser	Ser	Ser	Pro	Cys	Ser	Asn
				405					410					415	
Gly	Ala	Lys	Cys	Val	Asp	Leu	Gly	Asp	Ala	Tyr	Leu	Cys	Arg	Cys	Gln
			420					425					430		
Ala	Gly	Phe	Ser	Gly	Arg	His	Cys	Asp	Asp	Asn	Val	Asp	Asp	Cys	Ala
		435					440					445			
Ser	Ser	Pro	Cys	Ala	Asn	Gly	Gly	Thr	Cys	Arg	Asp	Gly	Val	Asn	Asp

FIGURE 4A

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450						455						460					
Phe	Ser	Cys	Thr	Cys	Pro	Pro	Gly	Tyr	Thr	Gly	Arg	Asn	Cys	Ser	Ala		
465						470				475					480		
Pro	Val	Ser	Arg	Cys	Glu	His	Ala	Pro	Cys	His	Asn	Gly	Ala	Thr	Cys		
				485					490					495			
His	Glu	Arg	Gly	His	Gly	Tyr	Val	Cys	Glu	Cys	Ala	Arg	Gly	Tyr	Gly		
			500					505					510				
Gly	Pro	Asn	Cys	Gln	Phe	Leu	Leu	Pro	Glu	Leu	Pro	Pro	Gly	Pro	Ala		
		515					520					525					
Val	Val	Asp	Leu	Thr	Glu	Lys	Leu	Glu	Gly	Gln	Gly	Gly	Pro	Phe	Pro		
		530				535					540						
Trp	Val	Ala	Val	Cys	Ala	Gly	Val	Ile	Leu	Val	Leu	Met	Leu	Leu	Leu		
545					550					555					560		
Gly	Cys	Ala	Ala	Val	Val	Val	Cys	Val	Arg	Leu	Arg	Leu	Gln	Lys	His		
				565					570					575			
Arg	Pro	Pro	Ala	Asp	Pro	Cys	Arg	Gly	Glu	Thr	Glu	Thr	Met	Asn	Asn		
			580					585					590				
Leu	Ala	Asn	Cys	Gln	Arg	Glu	Lys	Asp	Ile	Ser	Val	Ser	Ile	Ile	Gly		
		595					600					605					
Ala	Thr	Gln	Ile	Lys	Asn	Thr	Asn	Lys	Lys	Ala	Asp	Phe	His	Gly	Asp		
		610				615					620						
His	Ser	Ala	Asp	Lys	Asn	Gly	Phe	Lys	Ala	Arg	Tyr	Pro	Ala	Val	Asp		
625					630					635					640		
Tyr	Asn	Leu	Val	Gln	Asp	Leu	Lys	Gly	Asp	Asp	Thr	Ala	Val	Arg	Asp		
				645					650					655			
Ala	His	Ser	Lys	Arg	Asp	Thr	Lys	Cys	Gln	Pro	Gln	Gly	Ser	Ser	Gly		
		660						665					670				
Glu	Glu	Lys	Gly	Thr	Pro	Thr	Thr	Leu	Arg	Gly	Gly	Glu	Ala	Ser	Glu		
		675					680					685					
Arg	Lys	Arg	Pro	Asp	Ser	Gly	Cys	Ser	Thr	Ser	Lys	Asp	Thr	Lys	Tyr		
		690				695					700						
Gln	Ser	Val	Tyr	Val	Ile	Ser	Glu	Glu	Lys	Asp	Glu	Cys	Val	Ile	Ala		
705					710					715					720		
Thr	Glu	Val															

FIGURE 4A CONT

GAATTCCATT	TTAAGTTATA	CAAAACTGAT	TACCATAAGT	GCGGTCGACT	GCTTTTATTT	60
TTACGTTGTG	TGTGTTGGAA	AAATGCTAAA	ACATCAGTCT	ACAATTCTAT	ATATTGTTAT	120
TAAAGATTAA	TCCAACACGC	AACCCAAGGA	CATATAAGCG	ATTTCCACTA	TTGCATCAGA	180
GCACTCGGCA	GGAAAGGCCT	AGCCACGGGG	AACATTAGAA	GCTACAGAAG	CATTGCAGAG	240
AAGAGAAGAT	CCCCCGCGCG	TCCGCCGCTG	TTCTAAGGAG	AGAAGTGGGG	GCCCCCAGG	300
CTCGCGCGTG	GAGCGAAGCA	GCATGGGCG	TCCGTCGCG	CTGGCCCTGG	CGGTGCTCTC	360
GGCCTTGCTG	TGTCAGGTCT	GGAGCTCTGG	GGTGTTCGAA	CTGAAGCTGC	AGGAGTTCGT	420
CAACAAGAAG	GGGCTGCTGG	GGAACCGCAA	CTGCTGCCGC	GGGGGCGCGG	GGCCACCGCC	480
GTGCGCCTGC	CGGACCTTCT	TCCGCGTGTG	CCTCAGCAC	TACCAGGCCA	GCGTGTCCCC	540
CGAGCCGCCC	TGCACCTACG	GCAGCGCCGT	CACCCCGTGT	CTGGGCGTCG	ACTCCTTCAG	600
TCTGCCCGAC	GGCGGGGGCG	CCGACTCCGC	CTACGGAGAG	CCCATCCGCT	TCCCCTTCGG	660
CTTCACCTGG	CCGGGACACCT	TCTCTCTGAT	TATTGAAGCT	CTCCACACAG	ATTCTCCTGA	720
TGACCTCGCA	ACAGAAAACC	CAGAAAGACT	CATCAGCCGC	CTGGCCACCC	AGAGGCACCT	780
GACGGTGGGC	GAGGAGTGGT	CCCAGGACCT	GCACAGCAGC	GGCCGACCGG	ACCTCAAGTA	840
CTCCTACCCG	TTCTAGTAGT	ACGAACTACT	CTACGGAGAG	GGCTGCTCCG	TTTTCTGCCG	900
TCCCCGGGAC	GATGCCTTCG	GCCACTTCAC	CTGTGGGGAG	CGTGGGGAGA	AAGTGTGCAA	960
CCCTGGCTGG	AAAGGGCCCT	ACTGCACAGA	GCCGATCTGC	CTGCCTGGAT	GTGATGAGCA	1020
GCATGGATTT	TGTGACAAAC	CAGGGGAATG	CAAGTGCAGA	GTGGGCTGGC	AGGGCGAGTA	1080
CTGTGACGAG	TGTATCCAGT	ATCCAGGCTG	TCTCCATGGC	ACCTGCCAGC	AGCCCTGGCA	1140
GTGCAACTGC	CAGGAAGGCT	GGGGGGGCCCT	TTTCTGCAAC	CAGGACCTGA	ACTACTGCAC	1200
ACACCATAAG	CCCTGCAGA	ATGGAGCCAC	CTGCACCAAC	ACGGGCCAGG	GGAGCTACAC	1260
TTGCTCTTGC	CGGCCCTGGT	ACACAGGTGC	CACCTGCGAG	CTGGGGATTG	ACGAGTGTGA	1320
CCCCTAGCCG	TGTAGAACG	GAGGGAGCTG	GACGGATCTC	GAGAACAGCT	ACTCCTGTAC	1380
CTGCCCCACC	GGCTTCTACG	GCAAAATCTG	TGAATTGAGT	GCCATGACCT	GTGCGGACGG	1440
CCCTTGCTTT	AACGGGGGTC	GGTGCTCAGA	CAGCCCCGAT	GGAGGGTACA	GCTGCCGCTG	1500
CCCCGTGGGC	TACTCCGGCT	TCAACTGTGA	GAAGAAAATT	GACTACTGCA	GCTCTTCACC	1560
CTGTTCTAAT	GGTGCCCAAG	GTGTGGACCT	CGGTGTGCCC	TACCTGTGCC	GCTGCCAGGC	1620
CGGCTTCTCG	GGGAGGCACT	GTGACGACAA	CGTGGACGAC	TGCGCCTCCT	CCCCGTGCGC	1680
CAACGGGGGG	ACCTGCCCGG	ATGGCGTGAA	CGACTTCTCC	TGCACCTGCC	CGCCTGGCTA	1740
CACGGGCAGG	AACCTGCAGT	CCCCCGTCAG	CAGGTGCGAG	CACGCACCCT	GCCACAATGG	1800
GGCCACCTGC	CACGAGAGGG	GCCACGGCTA	TGTGTGCGAG	TGTGCCCGAG	GCTACGGGGG	1860
TCCCAACTGC	CAGTTCCCTG	TCCCCGAGCT	GCCCCCGGGC	CCAGCGGTGG	TGGACCTCAC	1920
TGAGAAGCTA	GAGGGCCAGG	GCGGGCCATT	CCCCGTGGGT	GCCGTGTGCG	CCGGGGTCAT	1980
CCTTGTCTCT	ATGCTGCTGC	TGGGCTGTGC	CGCTGTGGTG	GTCTGCGTCC	GGCTGAGGCT	2040
GCAGAAGCAC	CGGCCCCCAG	CCGACCCCTG	CCGGGGGGAG	ACGGAGACCA	TGAACAACCT	2100
GGCCAACCTG	CAGCGTGAGA	AGGACATCTC	AGTCAGCATC	ATCGGGGCCA	CGCAGATCAA	2160
GAACACCAAC	AAGAAGGCGG	ACTTCCACGG	GGACCACAGC	GCCGACAGA	ATGGCTTCAA	2220
GGCCCGCTAC	CCAGCGGTGG	ACTATAACCT	CGTGCAGGAC	CTCAAGGGTG	ACGACACCGC	2280
CGTCAGGGAC	GCGCACAGCA	AGCGTGACAC	CAAGTGCCAG	CCCCAGGGCT	CCTCAGGGGA	2340
GGAGAAGGGG	ACCCCGACCA	CACTCAGGGG	TGGAGAAGCA	TCTGAAAGAA	AAAGGCCGGA	2400
CTCGGGCTGT	TCAACTTCAA	AAGACACCAA	GTACCAGTCG	GTGTACGTCA	TATCCGAGGA	2460
GAAAGATGAG	TGCGTCATAG	CAACTGAGGT	GTAAAATGGA	AGTGAGATGG	CAAGACTCCC	2520
GTTTCTCTTA	AAATAAGTAA	AATTCCAAGG	ATATATGCC	CAACGAATGC	TGCTGAAGAG	2580
GAGGGAGGCC	TCGTGGACTG	CTGCTGAGAA	ACCGAGTTCA	GACCGAGCAG	GTTCTCCTCC	2640
TGAGGTCTCT	GACGCCTGCC	GACAGCCTGT	CGCGGCCCGG	CCGCCTGCGG	CACCTGCCTT	2700
CGTGACGTGC	CCGTTGCACT	ATGGACAGTT	GCTCTTAAGA	GAATATATAT	TTAAATGGGT	2760
GAACTGAATT	ACGCATAAGA	AGCATGCACT	GCCTGAGTGT	ATATTTTGGA	TTCTTATGAG	2820
CCAGTCTTTT	CTTGAATTAG	AAACACAAAC	ACTGCCTTTA	TTGTCCTTTT	TGATACGAAG	2880
ATGTGCTTTT	TCTAGATGGA	AAAGATGTGT	GTTATTTTTT	GGATTTGTAA	AAATATTTTT	2940
CATGATATCT	GTAAAGCTTG	AGTATTTTGT	GATGTTTCGT	TTTTATAATT	TAAATTTTGG	3000
TAAATATGTA	CAAAGGCACT	TCGGGCTCAT	GTGACTATAT	TTTTTTGTAT	ATAAATGTAT	3060
TTATGGAATA	TTGTGCAAA	GTTATTTGAG	TTTTTTACTG	TTTTGTTAAT	GAAGAAATTC	3120
CTTTTTAAAA	TATTTTTCCA	AAATAAATTT	TATGAGGAAT	TC		3162

FIGURE 4B

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Gly	Glu	Glu	Gly	Ser	Phe	Ser	His	Gly	Ser	Val	Ile	Asp	Gly	Arg	Phe
1				5					10					15	
Glu	Gly	Phe	Ile	Gln	Thr	Arg	Gly	Gly	Thr	Phe	Tyr	Val	Glu	Pro	Ala
			20					25					30		
Glu	Arg	Tyr	Ile	Lys	Asp	Arg	Thr	Leu	Pro	Phe	His	Ser	Val	Ile	Tyr
		35					40					45			
His	Glu	Asp	Asp	Ile	Ser	Glu	Arg	Leu	Lys	Leu	Arg	Leu	Arg	Lys	Leu
	50					55					60				
Met	Ser	Leu	Glu	Leu	Trp	Thr	Ser	Cys	Cys	Leu	Pro	Cys	Ala	Leu	Leu
65					70				75					80	
Leu	His	Ser	Trp	Lys	Ala	Val	Asn	Ser	His	Cys	Leu	Tyr	Phe	Lys	Lys
			85					90					95		
Asp	Phe	Trp	Gly	Phe	Ser	Glu	Ile	Tyr	Tyr	Pro	His	Lys	Tyr	Gly	Pro
			100					105					110		
Gln	Gly	Gly	Cys	Ala	Asp	His	Ser	Val	Phe	Glu	Arg	Met	Arg	Lys	Tyr
		115						120				125			
Gln	Met	Thr	Gly	Val	Glu	Glu	Val	Thr	Gln	Ile	Pro	Gln	Glu	Glu	His
	130					135					140				
Ala	Ala	Asn	Gly	Pro	Glu	Leu	Leu	Arg	Lys	Arg	Arg	Thr	Thr	Ser	Ala
145					150					155					160
Glu	Lys	Asn	Thr	Cys	Gln	Leu	Tyr	Ile	Gln	Thr	Asp	His	Leu	Phe	Phe
			165						170					175	
Lys	Tyr	Tyr	Gly	Thr	Arg	Glu	Ala	Val	Ile	Ala	Gln	Ile	Ser	Ser	His
			180					185					190		
Val	Lys	Ala	Ile	Asp	Thr	Ile	Tyr	Gln	Thr	Thr	Asp	Phe	Ser	Gly	Ile
		195					200					205			
Arg	Asn	Ile	Ser	Phe	Met	Val	Lys	Arg	Ile	Arg	Ile	Asn	Thr	Thr	Ala
	210					215					220				
Asp	Glu	Lys	Asp	Pro	Thr	Asn	Pro	Phe	Arg	Phe	Pro	Asn	Ile	Ser	Val
225					230					235					240
Glu	Lys	Phe	Leu	Glu	Leu	Asn	Ser	Glu	Gln	Asn	His	Asp	Asp	Tyr	Cys
			245						250					255	
Leu	Ala	Tyr	Val	Phe	Thr	Asp	Arg	Asp	Phe	Asp	Asp	Gly	Val	Leu	Gly
			260					265					270		
Leu	Ala	Trp	Val	Gly	Ala	Pro	Ser	Gly	Ser	Ser	Gly	Gly	Ile	Cys	Glu
		275						280				285			
Lys	Ser	Lys	Leu	Tyr	Ser	Asp	Gly	Lys	Lys	Lys	Ser	Leu	Asn	Thr	Gly
	290					295					300				
Ile	Ile	Thr	Val	Gln	Asn	Tyr	Gly	Ser	His	Val	Pro	Pro	Lys	Val	Ser
305					310					315					320
His	Ile	Thr	Phe	Ala	His	Glu	Val	Gly	His	Asn	Phe	Gly	Ser	Pro	His
			325						330					335	
Asp	Ser	Gly	Thr	Glu	Cys	Thr	Pro	Gly	Glu	Ser	Lys	Asn	Leu	Gly	Gln
			340					345					350		
Lys	Glu	Asn	Gly	Asn	Tyr	Ile	Met	Tyr	Ala	Arg	Ala	Thr	Ser	Gly	Asp
			355				360					365			
Lys	Leu	Asn	Asn	Asn	Lys	Phe	Ser	Leu	Cys	Ser	Ile	Arg	Asn	Ile	Ser
	370					375					380				
Gln	Val	Leu	Glu	Lys	Lys	Arg	Asn	Asn	Cys	Phe	Val	Glu	Ser	Gly	Gln
385					390					395					400
Pro	Ile	Cys	Gly	Asn	Gly	Met	Val	Glu	Gln	Gly	Glu	Glu	Cys	Asp	Cys
			405						410					415	
Gly	Tyr	Ser	Asp	Gln	Cys	Lys	Asp	Glu	Cys	Cys	Phe	Asp	Ala	Asn	Gln
			420					425					430		
Pro	Glu	Gly	Arg	Lys	Cys	Lys	Leu	Lys	Pro	Gly	Lys	Gln	Cys	Ser	Pro
		435					440					445			
Ser	Gln	Gly	Pro	Cys	Cys	Thr	Ala	Gln	Cys	Ala	Phe	Lys	Ser	Lys	Ser
	450					455					460				

FIGURE 3A

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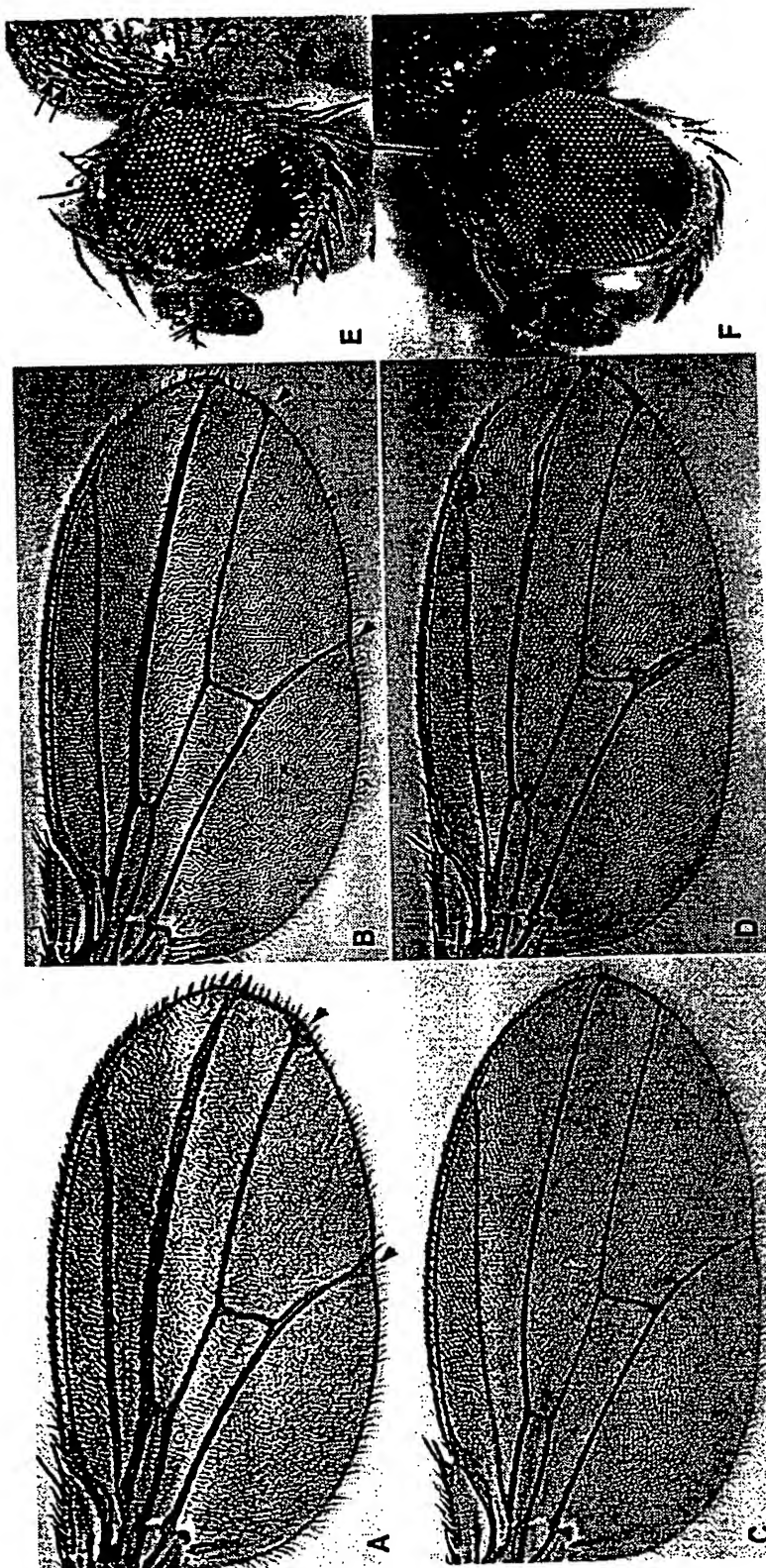
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 500 505 510
 Ser Ile Cys Glu Lys Tyr Gly Leu Glu Glu Cys Thr Cys Ala Ser Ser
 515 520 525
 Asp Gly Lys Asp Asp Lys Glu Leu Cys His Val Cys Cys Met Lys Lys
 530 535 540
 Met Asp Pro Ser Thr Cys Ala Ser Thr Gly Ser Val Gln Trp Ser Arg
 545 550 555 560
 His Phe Ser Gly Arg Thr Ile Thr Leu Gln Pro Gly Ser Pro Cys Asn
 565 570 575
 Asp Phe Arg Gly Tyr Cys Asp Val Phe Met Arg Cys Arg Leu Val Asp
 580 585 590
 Ala Asp Gly Pro Leu Ala Arg Leu Lys Lys Ala Ile Phe Ser Pro Glu
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 Leu Tyr Glu Asn Ile Ala Glu Trp Ile Val Ala His Trp Trp Ala Val
 610 615 620
 Leu Leu Met Gly Ile Ala Leu Ile Met Leu Met Ala Gly Phe Ile Lys
 625 630 635 640
 Ile Cys Ser Val His Thr Pro Ser Ser Asn Pro Lys Leu Pro Pro Pro
 645 650 655
 Lys Pro Leu Pro Gly Thr Leu Lys Arg Arg Arg Pro Pro Gln Pro Ile
 660 665 670
 Gln Gln Pro Gln Arg Gln Arg Pro Arg Glu Ser Tyr Gln Met Gly His
 675 680 685
 Met Arg Arg
 690

FIGURE 5A CONT

GGTGAAGAAG	GAAGTTTTAG	CCATGGGTCT	GTTATTGATG	GAAGATTTGA	AGGATTCATC	60
CAGACTCGTG	GTGGCACATT	TTATGTTGAG	CCAGCAGAGA	GATATATTAA	AGACCGAACT	120
CTGCCATTTT	ACTCTGTCAT	TTATCATGAA	GATGATATTA	GTGAAAGGCT	TAAACTGAGG	180
CTTAGAAAAC	TTATGTCACT	TGAGTTGTGG	ACCTCCTGTT	GTTTACCCTG	TGCTCTTCTG	240
CTTCACTCAT	GGAAGAAAGC	TGTAAATTCT	CACTGCCTTT	ACTTCAAGGA	TTTCTGGGGC	300
TTTTCTGAAA	TCTACTATCC	CCATAAATAC	GGTCTCAGG	GCGGCTGTGC	AGATCATTCA	360
GTATTTGAAA	GAATGAGGAA	ATACCAGATG	ACTGGTGTAG	AGGAAGTAAC	ACAGATACCT	420
CAAGAAGAAC	ATGCTGCTAA	TGGTCCAGAA	CTTCTGAGGA	AAAGACGTAC	AACTTCAGCT	480
GAAAAAATA	CTTGTCAGCT	TTATATTTCAG	ACTGATCATT	TGTTCTTTAA	ATATTACGGA	540
ACACGAGAAG	CTGTGATTGC	CCAGATATCC	AGTCATGTTA	AAGCGATTGA	TACAATTTC	600
CAGACCACAG	ACTTCTCCGG	AATCCGTAAC	ATCAGTTTCA	TGGTGAAACG	CATAAGAATC	660
AATACAACCTG	CTGATGAGAA	GGACCCTACA	AATCCTTTCC	GTTTCCCAAA	TATTAGTGTG	720
GAGAAGTTTC	TGGAATTGAA	TTCTGAGCAG	AATCATGATG	ACTACTGTTT	GGCCTATGTC	780
TTACAGACAC	GAGATTTTGA	TGATGGCGTA	CTTGGTCTGG	CTTGGGTTGG	AGCACCTTCA	840
GGAACTCTTG	GAGGAATATG	TGAAAAAAGT	AAACTCTATT	CAGATGGTAA	GAAGAAGTCC	900
TTAAACACTG	GAATTATTAC	TGTTCAGAAC	TATGGGTCTC	ATGTACCTCC	CAAAGTCTCT	960
CACATTACTT	TTGCTCACGA	AGTTGGACAT	AACTTTGGAT	CCCCACATGA	TTCTGGAACA	1020
GAGTGCACAC	CAGGAGAATC	TAAGAATTTG	GGTCAAAAAG	AAAATGGCAA	TTACATCATG	1080
TATGCAAGAG	CAACATCTGG	GGACAAACTT	AACAACAATA	AATTCTCACT	CTGTAGTATT	1140
AGAAATATAA	GCCAAGTTCT	TGAGAAGAAG	AGAAACAAC	GTTTTGTTGA	ATCTGGCCAA	1200
CCTATTTGTG	GAAATGGAAT	GGTAGAACAA	GGTGAAGAAT	GTGATTGTGG	CTATAGTGAC	1260
CAGTGTAAG	ATGAATGCTG	CTTCGATGCA	AATCAACCAG	AGGGAAGAAA	ATGCAAACTG	1320
AAACCTGGGA	AACAGTGCAG	TCCAAGTCAA	GGTCTTTGTT	GTACAGCACA	GTGTGCATTG	1380
AAGTCAAAAGT	CTGAGAAGTG	TCGGGATGAT	TCAGACTGTG	CAAGGGAAGG	AATATGTAAT	1440
GGCTTCACAG	CTCTCTGCCC	AGCATCTGAC	CCTAAACCAA	ACTTCACAGA	CTGTAATAGG	1500
CATACACAAG	TGTGCATTAA	TGGGCAATGT	GCAGGTTCTA	TCTGTGAGAA	ATATGGCTTA	1560
GAGGAGTGTA	CGTGTGCCAG	TTCTGATGGC	AAAGATGATA	AAGAATTATG	CCATGTATGC	1620
TGTATGAAGA	AAATGGACCC	ATCAACTTGT	GCCAGTACAG	GGTCTGTGCA	GTGGAGTAGG	1680
CAC TTCAGTG	GTCGAACCAT	CACCCTGCAA	CCTGGATCCC	CTTGCAACGA	TTTTAGAGGT	1740
TACTGTGATG	TTTTTCATGCG	GTGCAGATTA	GTAGATGCTG	ATGGTCCTCT	AGCTAGGCTT	1800
AAAAAAGCAA	TTTTTAGTCC	AGAGCTCTAT	GAAAACATTG	CTGAATGGAT	TGTGGCTCAT	1860
TGGTGGGCAG	TATTACTTAT	GGGAATTGCT	CTGATCATGC	TAATGGCTGG	ATTTATTAAAG	1920
ATATGCAGTG	TTCATACTCC	AAGTAGTAAT	CCAAAGTTGC	CTCCTCCTAA	ACCACTTCCA	1980
GGCACTTTAA	AGAGGAGGAG	ACCTCCACAG	CCCATTGAGC	AACCCAGCG	TCAGCGGCCC	2040
CGAGAGAGTT	ATCAAATGGG	ACACATGAGA	CGCTAACTGC	AGCTTTTGCC	TTGGTTCTTC	2100
CTAGTGCCTA	CAATGGGAAA	ACTTCACTCC	AAAGAGAAAC	CTATTAAAGTC	ATCATCTCCA	2160
AACTAAACCC	TCACAAGTAA	CAGTTGAAGA	AAAAATGGCA	AGAGATCATA	TCCTCAGACC	2220
AGGTGGAATT	ACTTAAATTT	TAAAGCCTGA	AAATTTCCAAT	TTGGGGGTGG	GAGGTGGAAA	2280
AGGAACCCAA	TTTTCTTATG	AACAGATATT	TTTAACTTAA	TGGCACAAAG	TCTTAGAATA	2340
TTATTATGTG	CCCCGTGTTT	CCTGTTCTTC	GTTGCTGCAT	TTTCTTCACT	TGCAGGCAAA	2400
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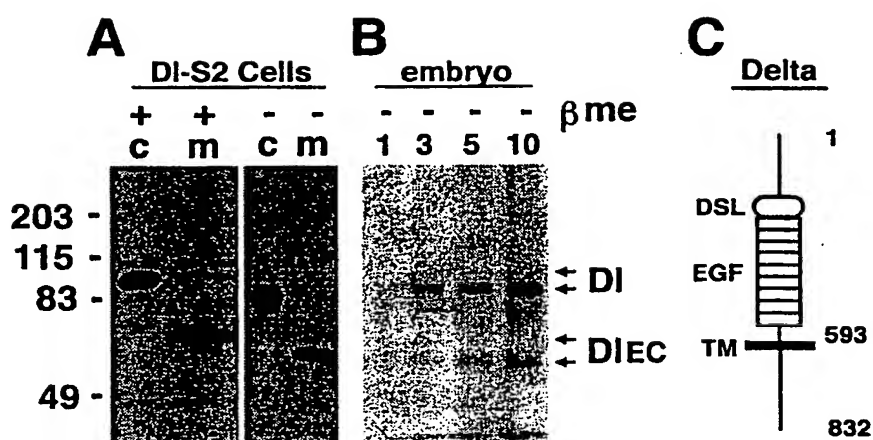
FIGURE 5B

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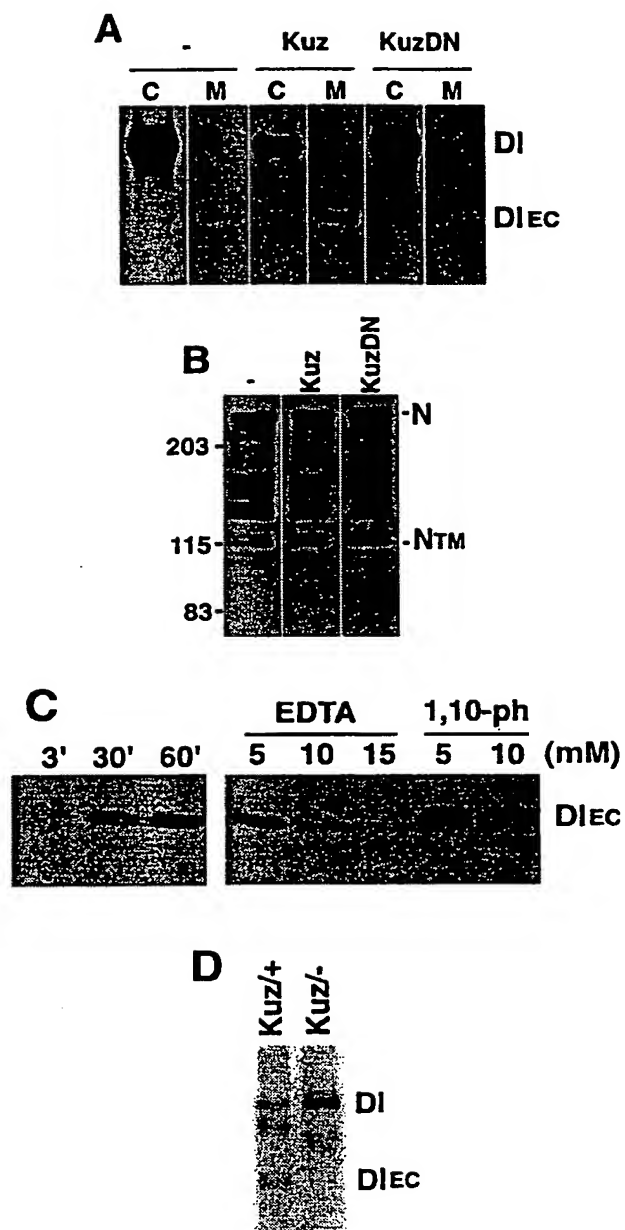
FIGURES 6A-6F

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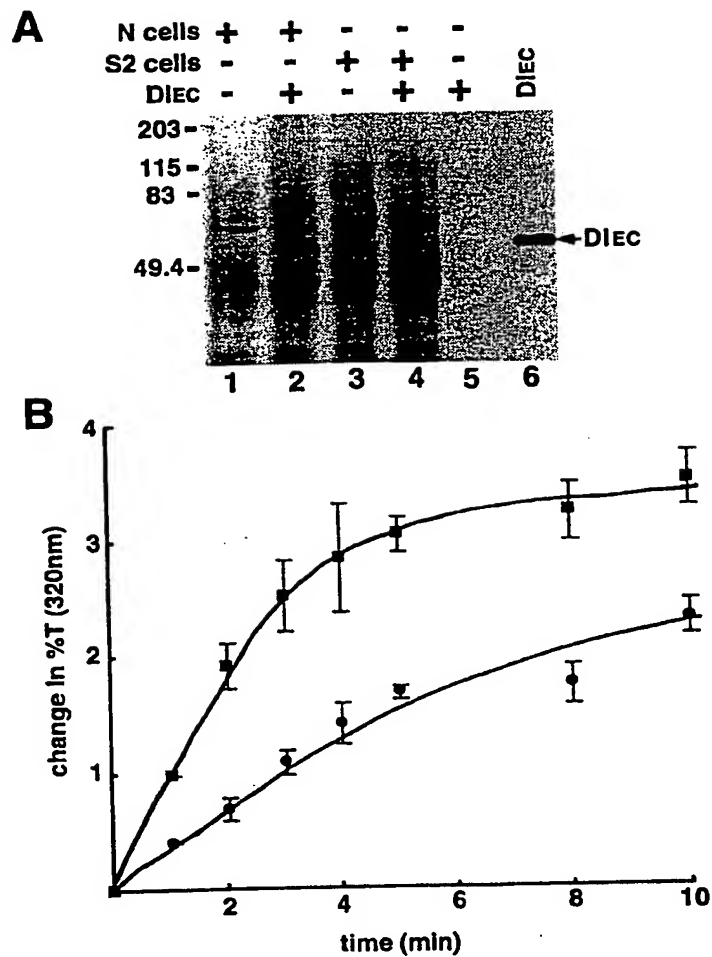
FIGURES 7A-7C

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FIGURES 8A-8D

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FIGURES 9A-9B

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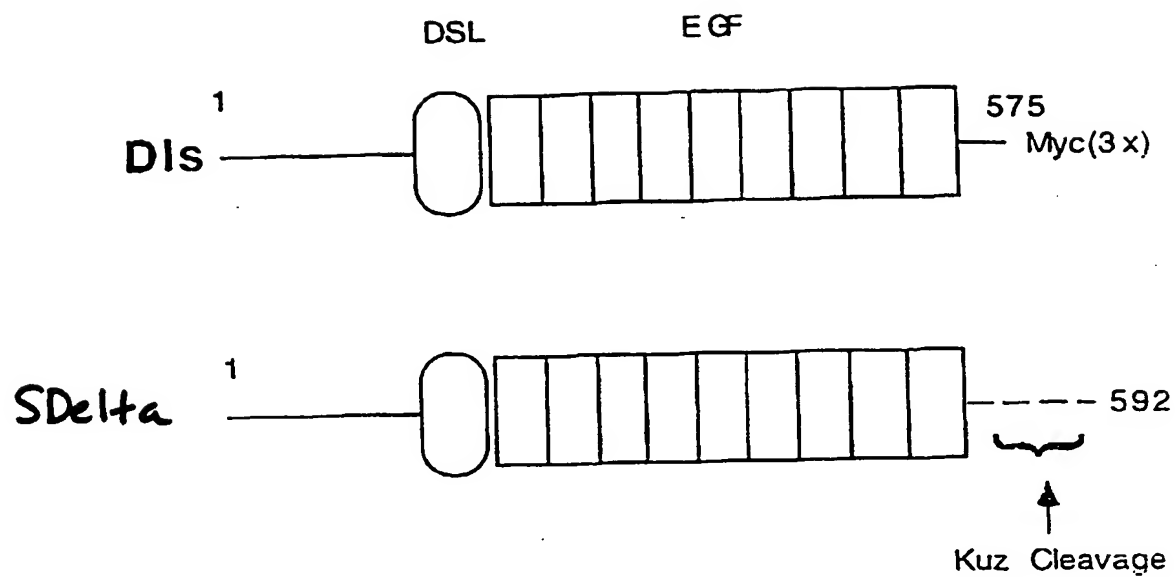


FIGURE 10

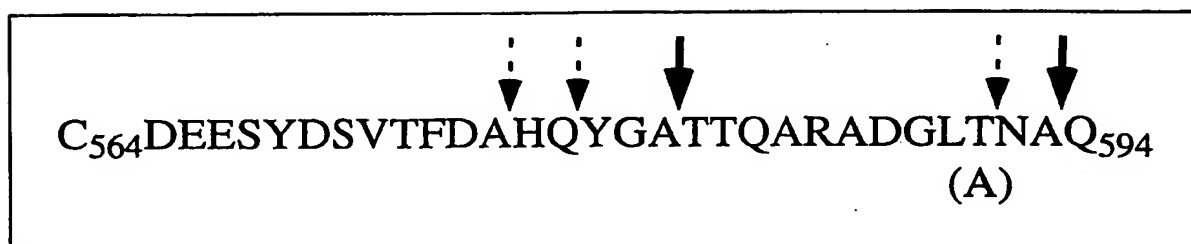


FIGURE 11